Lead poisoning in swans Cygnus olor

Thesis

Lead Poisoning in Swans *Cygnus olor*

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To Duchess

I would give anything to
live those years again.

Abstract

This study examines some aspects of lead poisoning in the Mute Swan (Cygnus olor). In 4 years I examined 860 carcasses from a study area in East Anglia. Seventy-four per cent of the birds concerned had died of lead poisoning, from ingested fishing weights. Annual aerial surveys showed that the number of live birds in the study area declined by 22% during the 4 year period. When 23 carcasses were re-introduced to their original locations, 5 were re-notified and 16 disappeared without trace. A comparison of gizzard grit size between a group of lead-poisoned birds and a similar number of birds, which had died of other causes, indicated that lead weights, when available are ingested by choice. Results from an environmental survey showed that without ingesting fishing weights swans carry a light burden of lead, resulting from contamination by airborne lead, probably originating from petrol-engined motor vehicles.

An analysis using the bodies of lead-poisoned swans, found freshly dead in the field, showed that lead can be excreted via the bile and lower gut.

Controlled experiments using pigeons (Columba livia) and mallard (Anas platyrhynchos) showed that:- (1) tissue lead and zinc values monitored over a period of one year became elevated due to changes in physiology during moult; and (2) changes in calcium metabolism during eggshell formation had no detectable effect on blood lead values. Results indicated that in pigeons most of the calcium used in the formation of eggshells is derived directly from the diet, rather than from bone reserves.

As a treatment for lead poisoning, daily injections of thiamin were administered to lead-poisoned mallard. No detectable effect on the progress of the intoxication was found.

Tissue lead values were lower in lead-dosed ducks receiving a diet supplemented with phytic acid than in a similar group receiving no supplementation.

Pigeons dosed with lead scored more "fails" during a simple performance test which measured landing ability than did a control group of birds.

Zinc was tested as a possible fishing weight substitute and, at the levels administered, was found to be non-toxic to mallard. Combined dosing of lead and zinc showed that zinc had a demonstratable protective action against lead toxicity.

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C O N T E N T S

Introduction

Lead poisoning in Mute Swans (Cygnus olor), due to the ingestion of lead fishing weights, was first recognised in 1973 (Simpson et al 1979). Since that date post-mortem and blood lead examinations from various parts of Britain have shown lead poisoning to be a major mortality factor in Mute Swans (Birkhead 1982, 1983; French 1982; Sears 1988, 1989).

In this present study I chose to concentrate on some aspects of lead poisoning which could be investigated by post-mortem examination of dead swans and by controlled laboratory experiments using other animals. I concentrated on those aspects of the problem which had been least thoroughly studied by others, and on which information was most needed.

The investigation is divided into 10 parts, each written as a distinct chapter. Relevant references follow each chapter. Two chapters have already been published as scientific papers.

Swan carcasses which were collected from my study area, East Anglia, were used to determine the proportion of swans dying of lead poisoning (Chapter 2). The fresh bodies of swans found dead in the field were used in the investigations reported in Chapter 5. For the rest of the study, ducks (Chapter 6, 7, 9 and 10) and pigeons (Chapter 3, 4 and 8) were used as experimental animals.

To complete this study, I have driven over 28,000 miles, carried out approximately 1000 post-mortem examinations and processed a similar number of X-ray plates. Together with Paul Freestone, I have analysed over 3500 tissues for 4000 determinands.

The aims of the study were as follows:-

1) To investigate the proportion of swan deaths in a defined study area caused by the ingestion of lead fishing weights.

2) To investigate seasonal changes in liver lead residues.

3) To determine the effect of calcium stress during egg formation on blood lead values.

4) To investigate the role which bile and gut secretions play in the elimination of lead from the bodies of lead poisoned swans.

5) To study the effect of daily administration of Thiamin (Vitamin B1) on lead poisoned ducks.

6) To determine what effect a diet supplemented with sodium phytate has on lead toxicity and tissue lead residues in ducks.

7) To investigate what effect dosing pigeons with lead shot has on motor co-ordination and landing ability.

8) To investigate the suitability of zinc as an alternative to lead as a fishing weight and gunshot.

9) To study the effect of combined dosing of both zinc and lead to mallard.

Chapter 1

Background

Throughout history lead and its salts have caused considerable human and animal suffering. The indiscriminate use of lead salts has had horrifying consequences such as white lead added to flour to make a whiter loaf, and lead acetate or sugar of lead added to wine to make it more palatable. Such practices were only prohibited under the Preservatives Regulations 1925-27 when it became illegal to use any compound of lead in food.

There have also been many unusual causes of lead poisoning in people including the use of old car batteries as a regular source of fuel, lead opium pipes, theatrical grease paint containing lead pigments and the use of lead paint in domestic accommodation and on childrens toys (Curry 1964).

Lead is the most common cause of accidental poisoning in domestic animals (Waldron & Stöfen 1974). Dogs (Zook et al. 1972), cattle (Hammond et al. 1956), and sheep (Allcroft & Blaxter 1950) have all been poisoned. Primates, fruit bats and other animals in zoos have become intoxicated with lead after chewing bars painted with lead based paints (Zook et al. 1970). Atmospheric fallout of lead has also caused lead poisoning in zoo animals (Bazell 1971).

Waterfowl ingest spent lead gunshot during feeding and retain it as grit in the gizzard; as the pellets are eroded, some of the lead is rendered soluble and is absorbed in the digestive tract. Lead intoxication follows and the birds may eventually die. This form of lead poisoning has been known in wild waterfowl since 1874 (Phillips & Lincoln 1930). Wetmore (1919) predicted that lead poisoning due to ingestion of gunshot would increase, and from then to the present much scientific literature has been published on this topic. The reports vary in magnitude from "several mallard ducks dying of lead poisoning" to an "estimated 16,000 ducks dying in a period of two years". Based on his examination of 1003 ducks gizzards (Reid 1948) calculated that 225,000 ducks in the Minasota shooting bag contained lead shot. Bellrose (1959) documented 34 die-offs due to lead poisoning between 1937 and 1959.

The number of dead birds in these and other die-offs is not a reliable index of the impact of ingested lead shot. Continual low level mortality usually goes undetected in wild waterfowl populations. This was clearly shown by Bellrose (1959) when he dosed and tagged several thousand mallard (Anas platyrhynchos) with varying loads of lead shot. Birds dosed with only one lead shot were more vulnerable to hunting and had higher mortality in the first year than did controls. In the United States lead poisoning is thought to account for the loss of

of between 1.6 and 2.4 million ducks annually, a figure which excludes sublethally affected birds (Bellrose 1959, US Fish and Wildlife Service 1976), in Great Britain a survey by Mudge (1983) suspected that 8000 mallards are lost each year after ingesting lead shot.

During 1973 on the River Trent in Nottingham up to 17 Mute Swans (Cygnus olor) died of lead poisoning. The cause was found to be lost or discarded lead fishing weights (Simpson et al. 1979). In the following years several surveys demonstrated that there was a serious national lead poisoning problem for mute swans (Hunt, 1980; Birkhead, 1982; French 1982; Sears 1988).

The Nature Conservancy report (Lead Poisoning in Swans 1981) summarised the available evidence on lead poisoning in the British mute swan population. This report highlighted several areas of high risk where in some cases up to 80% of the swans examined had died of lead poisoning after ingesting lead fishing weights.

It is generally agreed that the ingestion by birds of lead shot or its salts has no beneficial effect and that lead at elevated levels plays no part in normal metabolic activities. But here the agreement almost ends.

Wetmore (1919) states that ducks with lead poisoning always had a good appetite, although six No.6 gunshot were nearly always fatal, whilst other authors (Bellrose 1964, Jordan & Bellrose 1951) observed that ducks cease to feed as lead intoxication progresses.

Young birds have been shown to be more susceptible than adults, and males more vulnerable than females in the breeding season, although the latter position was reversed in the autumn (Jordan & Bellrose 1951). No difference in susceptibility according to age or sex was shown with ducks dosed with eight No.6 lead shot (Longcore 1964).

The effect of diet on lead poisoning has been studied. Mallard on a whole corn diet dosed with gunshot experienced higher mortality than dosed birds which were fed on corn-meal (Jordan & Bellrose 1951). But other authors have demonstrated no difference (Longcore et al. 1964). Similarly, Irby et al. (1967) dosed mallard with 8 No.6 shot and found a 90-100% mortality in ducks on a whole corn diet. Birds dosed with 5 No.6 shot on a high fibre diet retained more shot and were clinically more ill than birds with the same dose on a low fibre diet. The high fibre diet also contained higher levels of selenium and calcium (Clemens et al. 1975). Supplementation of the diet with selenium reduced mortality of lead dosed mallards below that of birds on a low selenium diet (US Fish Wildlife Service 1976).

Grit status and type has been discussed by several authors. Birds which were deprived of grit but given lead shot withstood lead poisoning longer than birds provided with adequate supplies of grit (Wetmore 1919). The erosion of ingested lead shot was higher in birds provided with grit than in birds deprived of grit (Jordan 1952). Godin (1967) showed no difference between lead poisoned mallard provided with various types of grit or no grit at all. Longcore et al. (1964) found that dosed birds allowed access to crushed oystershell grit experienced fewer deaths than birds fed either quartz grit or no grit at all, and that lead dosed birds with grit experienced fewer deaths than lead dosed birds without grit. Beer & Stanley (1965) maintained that excess grit rapidly passes through the alimentary tract, taking any lead pellets with it.

Experiments with lead salts show equally conflicting results with different bird species. Ringed turtle doves, given lead acetate in drinking water had higher tissue levels of lead than did control birds (Kendall & Scanlon 1981), whereas broiler chickens given a similar treatment did not have significantly higher levels of lead than control birds (Vogt et al. 1977).

The pathology of lead poisoning has been extensively reported in various waterfowl (Wobeser 1981). Swans suffering from lead poisoning

can be recognised by the abnormal carriage of the neck, with the lower third usually supported on the back. (Simpson et al. 1979, Birkhead 1982). On land, affected birds are usually found lying down with the full length of the neck resting on the back or alongside the body on the ground. Affected birds in the final stages of lead poisoning are unable to move.

During the post-mortem examination of geese Adler (1944) observed ulceration of the gizzard epithelium and enlarged livers and spleens. In swans Simpson et al. (1979) did not observe such symtoms and livers and spleens were much smaller than those in control birds. Grey or green livers were noted in Canada Geese Branta canadensis by Cook and Trainer (1966), and in swans by Rosen and Bankowski (1960) and French (Chapter 2), but not by Simpson et al. (1979). Oedema of the head was noted by Trainer & Hunt (1965) and Bagley et al. (1967) in Whistling Swans Cygnus columbianus and Canada Geese Branta canadensis respectively, but not by French (Chapter 2) or Simpson et al. (1979) in mute swans.

Various haematological parameters have also been studied. (See Bates et al. 1968, Simpson et al. 1979, O'Halloran and Myers 1989).

REFERENCES

Adler, F. (1944). Chemical analysis of organs from lead poisoned Canada geese. J. Wildl. Mgmt. **8**, 83-5.

Allcroft & Blaxter (1950). Lead as a nutritional hazard to farm livestock. J. Comp. Path. Ther. **60**, 209.

Bagley, G.E., Locke, L.N. & Nightingale, G.T. (1967). Lead poisoning in Canada Geese in Delaware. Avian Dis. **11**, 608-8.

Bates, F.Y., Barnes, D.M. & Higbee, J.M. (1968). Lead toxicosis in mallard ducks. Bull. Wildl. Dis. Ass. **4**, 116-25.

Bazell (1971). Lead Poisoning: Zoo animals may be the first victims. Science **173**, 130-131.

Bellrose, F.C. (1959). Lead poisoning as a mortality factor in Waterfowl populations. Ill. Nat. Hist. Surv. Bull. **27(3)**, 235-288.

Bellrose, F.C. (1964). Spent shot and lead poisoning. In Waterfowl tomorrow. J.P. Linduska, US Government Printing Office, Washington DC.

Birkhead, M.E. (1982). Causes of mortality in the Mute Swan on the River Thames. J. Zool. Lond. **198**: 15-25.

Clausen, B. & Wolstrup, C. (1979). Lead poisoning in game from Denmark. Dan. Rev. Gam. Biolo. **11**: 1.

Clemens, E.T., Krook, L., Aronson, A.L. & Stevens, C.E. (1975). Pathogenesis of lead shot poisoning in the mallard duck. Cornell vet. **65**: 248-285.

Cook, R.S. & Trainer, D.O. (1966). Experimental lead poisoning of Canada Geese. J. Wildl. Mgmt. **30**, 1-8.

Curry, A.S. (1964). Methods of Forensic Science. Vol III. Interscience Publishers.

Drasch, G.A. (1982). Lead burden in prehistorical, historical and modern human bones. Science of the Total Environment **24**, 199-231.

Gilfillan, S.C. (1965). Lead poisoning and the fall of Rome. Journal of Occupational Medicine **7**, 53-60.

Godin, A.J. (1967). Test of grit types in alleviating lead poisoning in mallards. Bureau of Sport, Fisheries and Wildlife Spec. Sci. Rep. Wildl., 107. Washington DC.

Hammond, P.B., Wright, H.N. & Roepke, M.H. (1956). University of Minnesota Agricultural Experimental Station. Technical Bulletin No.221.

Irby, H.D., Locke, L.N. & Bagley, G.E. (1967). Relative toxicity of lead and selected substitute shot types to game farm mallards. J. Wildl. Manage. **31(2)**: 253-257.

Jordan, J.S. & Bellrose, F.C. (1951). Lead poisoning in Wild Waterfowl. Ill. Nat. Hist. Surv. Biol. Notes 26 27 p.

Jordan, J.S. (1952). Lead poisoning in migratory Waterfowl with special reference to the mallard, Anas platyrhynchos Linnaeus, Ph. D. Dissertation, University of Michigan, Ann Arbor.

Kendall, R.J. & Scanlon, P.F. (1981). Effects of chronic lead ingestion on reproductive characteristics of ringed turtle doves Streptopelia risoria and on tissue lead concentrations of adults and their progeny. Environ. Pollut. (Series A) **26**, 203-213.

Longcore, J.R., Andrews, R., Locke, L.N., Bagley, G.E. & Young, L.T. (1964). Toxicity of lead and proposed substitute shot to mallards Bureau of Sport, Fisheries and Wildlife Spec. Sci. Rep. Wildl., 183. Washington DC.

O'Halloran, J. & Myers, K.A. (1989). Some sub-lethal effects of lead on Mute Swans Cygnus olor. J. Zool. Lond. **218**, 627-632.

Phillips, J.C. & Lincoln, F.C. (1930). American Waterfowl Houghton Mifflin Co., Boston and New York.

Reid, V.H. (1948). Lead shot in Minisota Waterfowl. J. Wildl. Manage. **12(2)**: 123-127.

Rosen, M.N. & Barkowski, R.A. (1960). A diagnostic technique and treatment for lead poisoning in swans. Calif. Fish. Game **46**, 81-90.

Sears, J. (1988). Regional and seasonal variations in lead poisoning in the Mute Swan Cygnus olor in relation to the distribution of lead and lead weights in the Thames area, England. Biol. Cons. **46**, 115-134.

Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of Mute Swans. Environ. Pollut. **18**: 187-202.

Trainer, D.O. & Hunt, R.A. (1965). Lead poisoning in whistling swans in Wisconsin. Avian Dis. **9**, 252-64.

US Fish and Wildlife Service (1976). Final Environmental Statement. Proposed use of steel shot for Hunting Waterfowl in the limited states. US Dept. of Interior, Washington DC.

Vogt, H., Nezel, K. & Matthes, S. (1977). Nutrition and Metabolism **2**, 203-204.

Waldron, H.A. & Stöfen, D. (1974). Sub-clinical lead poisoning. Academic Press. ISBN 0-12-671650-1.

Wetmore, A. (1919). Lead poisoning in waterfowl US Department of Agriculture Bull., 793. Washington DC.

Wobeser, G. (1981). Diseases of wild waterfowl. Plenum Press ISBN 0-306-40746-7.

Zook, B.C., Sauer, R.M. & Garner, F.M. (1970). Lead poisoning in Australian Fruit Bats. J. Am. Vet. Ass. **157**, 691-694.

Zook, B.C., Carpenter, J.L. & Roberts, R.M. (1972). Lead poisoning in dogs: occurrence, source, clinical pathology and electro-encephalography. Am. J. Vet. Res. **33**, 891-902.

Chapter 2

Lead Poisoning in Mute Swans (*Cygnus olor*) A Survey in East Anglia from October 1981 to January 1986

INTRODUCTION

The poisoning of waterfowl by the ingestion of gun-shot pellets is well documented throughout Europe and North America (Belrose 1959; Del Bono 1970; Clausen et al. 1975; Beer & Stanley 1964; French 1982). In Britain, a joint study, carried out in 1973 by ITE and the Veterinary Investigation Service near Nottingham, was able to identify for the first time that ingested anglers' weights were responsible for the deaths of Mute Swans (Cygnus olor) on the River Trent in Nottingham (Simpson et al. 1979). In America, several common loons (Garia immer) died after ingesting lead anglers' weights (Locke et al. 1982). Swans and other waterfowl are assumed to take in the lead weights along with, or in mistake for, grit which they need to aid the breakdown of food in the gizzard. Following the joint ITE-VI Centre study in 1979, the Nature Conservancy Council report on lead poisoning in swans was published (NCC 1981). This report identified lead poisoning from anglers' weights as being the biggest single cause of recorded Mute Swan deaths in Great Britain. It also identified several 'hot spots', where 70-90% of reported swan deaths were due to lead poisoning. Other

studies have shown that previously substantial swan populations have declined markedly, (eg River Thames, Birkhead 1982), while in others they had disappeared almost completely (eg River Avon, near Stratford, NCC 1981). Further surveys on the Thames carried out regularly since then have shown a further decline (Sears 1988).

The major objectives of my study were:

(1) To carry out post-mortem examinations (a) to determine the proportion of recorded swan deaths attributable to the ingestion of lead fishing weights and (b) to examine the bodies for any pathological changes associated with lead intoxication.

(2) To census the swan population of the study area once each year to gain a measure of the proportion later found dead.

(3) To examine the content of lead in river water, soil, vegetation, fish and live swan blood, as a measure of environmental contamination.

(4) To examine the relationship between gizzard grit size and ingested lead weights in dead birds.

(5) To determine the reporting rate of dead swans by reintroducing swan carcasses back into the field.

Study Area

Between October 1981 and January 1986 a study was carried out on the rivers Welland, Nene, Ouse and Cam in East Anglia, a total river length

of approximately 500 km and also on gravel pits and reservoirs within the same area. During this time, 848 Mute Swan (Cygnus olor) carcases were acquired from the area and examined, together with 12 live birds which later died, an average of more than 200 per year.

METHODS

Post-mortem Analysis

Immediately after receipt, carcasses were X-rayed to facilitate easier location of lead fishing weights or other foreign objects. The contents of the gizzard and proventriculus were examined and pieces of lead shot were identified by eye. In the majority of birds with lead shot, it was possible to distinguish visually between fishing weights and spent gun-shot. Where there was doubt, the material was tested chemically to differentiate between the 2 sources. Gunshot lead contains high levels of antimony as a metal hardener, whereas fishing weights, which need to remain pliable, contain virtually no antimony. Only 14 mute swans were shown to have carried gun-shot in their gizzards. These birds were eliminated from the study, even though 5 contained anglers' weights as well as gun-shot. Marked swans which had received treatment for lead poisoning and were then introduced into the area by rescue services and subsequently died were also rejected. Most

of these swans originated from the Norfolk Broads and had already been included in data on lead poisoning produced by the now defunct Swan Rescue Service. A large flock of swans at St Neots on the River Ouse received regular homeopathic treatment by a local animal welfare group as a preventitive measure for lead poisoning. This treatment consisted of dosing the swans with bread soaked in extremely dilute solutions of lead. I was unable to find a way of determining what contribution this treatment would have had on tissue lead levels and so any bodies from this area were excluded from the survey. Chemical analyses of liver, kidney and bone were carried out to help establish the cause of death.

Pathology and Clinical Observations

Birds suffering from lead poisoning had an abnormal carriage of the neck, the lower third being supported against the back. Many birds in the later stages of lead intoxication become anorexic due to the impaction of the gizzard, proventriculus and oesophagus, with food unable to pass through the alimentary canal. The birds were invariably emaciated, exhibiting a 'razor keel'. Vent feathers were soiled and usually stained green. Many birds, even in this state, would carry on preening. Prior to death cloacal temperature would fall from 41°C to 38°C and remain low until death. Birds suffering from lead poisoning were usually easy to catch and handle.

At post-mortem, pectoral muscle, liver, kidneys and heart were found to be smaller than in birds dying of other causes. Livers were dark and metallic in appearance with green staining around the gall-bladder, which was always grossly distended. Kidneys were loose and were more easily removed from the body than those from healthy birds. Impaction of either the gizzard, proventriculus or oesophagus, or a combination of all three, occurred in 58% of those birds dying of lead poisoning. The horny epithelial lining of the gizzard was usually damaged and easy to remove. The gizzard invariably contained lead weights as well as food and grit. The intestines were flabby, devoid of food and had a putrified odour. The cloaca usually contained clear watery excrement.

These findings are in general agreement with previously reported data (Simpson et al. 1979; Birkhead 1982).

Aerial Survey

In order to assess the number of swans in the study area, an aerial survey was carried out in November each year during 1981-1984. The area covered the Rivers Welland, Nene and Ouse, a total river length of 500 km, together with associated gravel pits. Individual counts were completed within 7 hours on the same day, thus minimising the effects of movement.

Environmental Surveys of lead

An environmental survey was carried out in Peterborough along the River Nene, at sampling sites at various distances from, and including, the city centre (the main river bridge into the town). The presence of swans determined the sampling points and from each swan that could be caught a blood sample was taken. Water, soil above the river water level and bank vegetation (grass) were also sampled. A selection of fishing weights was also collected along the river bank.

Water samples

Samples of river water were taken just below the water surface. In order to remove suspended matter, samples were filtered within 4 hours and a 20 ml subsample was then acidified with 2 ml of concentrated nitric acid and stored at +4°C.

Soil samples

Soil samples were taken over an area of approximately 100 cm^2 to a depth of 5 cm. Samples were then air dried at room temperature to constant weight. After first removing large stones, samples were X-rayed to detect the presence of lead fishing weights, which were removed. The soil was then ground and that which passed through a 4 mm sieve was mixed and a 5 g subsample removed for chemical analysis.

Vegetation samples

Grass samples which were made up mainly of Lolium perenne, Agrostis tenuis and Poa pratensis were taken over an area of approximately 2500 cm^2, these were then air dried at room temperature to constant weight, mixed thoroughly and a 5 g sample taken for chemical analysis.

Fish samples

Seven fish samples were obtained from anglers along the river bank, and consisted of 3 Bream (Abramis brama), 2 Roach (Rutilus rutilus) and 2 Rudd (Scardinius erythrophthalamus). The fish were killed, and dissected. Five gram samples of muscle were removed and stored at -20°C until analysis.

Chemical analysis

Swan tissue samples taken during post-mortem investigation were oven-dried at 80°C to constant weight. All samples were then digested with 5 ml concentrated nitric acid at room temperature for 12 hours, after which the tube contents were boiled at 120°C until all dark brown fumes had ceased. After cooling, the digest was then made up to 25 ml with glass distilled water. This final digest was analysed for lead by atomic absorption spectrophotometry, using an IL 151 AA/AE Spectrophotometer. Results were quantified by comparison with lead standards purchased from British Drug Houses, Poole, Dorset. Samples

taken in the environmental survey and a number of fishing weights discovered in the same area were further analysed by Inductively Coupled Plasma Mass Spectrometry to determine lead isotope ratios. This method helped to characterise the lead by determining the ratio of the four different lead isotopes. Tissue exposed to a particular type of lead will reflect the same characteristic isotope ratio (Campbell & Delves 1989), and hence indicate the source of the lead.

Grit status

A comparison of the dry weight and size of gizzard grit was made between 50 lead poisoned swans and a similar number of swans which had died of other causes, mainly cable collisions and vandalism. Grit size was determined by shaking the grit through a tower of sieves of decreasing mesh sizes. The various fractions were then reweighed.

Carcass Recovery Experiment

Twenty three unmarked dead swans which were collected by me over a period of 16 days were X-rayed, and samples of liver, kidney and bone were removed for chemical analysis. The bodies were marked and stored in a cold room after first being cleaned and the post-mortem incision sutured. Forty-eight hours after the last bird was received, they were all returned to their original locations and revisited every 48 hours.

RESULTS

Cause of death

The majority of the swans (848) were corpses with no known history, but in 12 live birds clinical observations were available following X-ray and veterinary examination. The symptoms in these 12 birds were anorexia, weakness, inability to move correctly, impaction of the oesophagus and emaciation. In addition, a marked and sustained fall in body temperature, observed up to 72 hours prior to death, was noted in the 12 live birds. A similar drop in body temperature was demonstrated with pigeons and ducks dosed with lead fishing weights in the laboratory.

At post-mortem, sexes were determined and significantly more (68% 585) of the birds examined were female ($X^2 = 111.74$, 1 d.f., $P < 0.001$). The sexes and monthly receipts of swan bodies are shown in Figure 1.

For comparative purposes the swan mortality data were divided in four-monthly periods, November-February, March-June and July-October (Table 3). The March-June period includes the coarse fishing closed season and also most of the swans breeding season. The data show that disproportionaly more birds died from lead poisoning during the periods November-June than during July-October. No great decline in lead-

induced deaths was apparent in March-June, but the lack of fishing then may have contributed to reduced lead mortality in the following period.

Lead shot was present in the alimentary tract of 562 swans and in all such birds, death was due to lead poisoning. Fishing hooks, with or without attached line, were found in 19 birds and in only 3 could these have contributed to death; nine such birds died of lead poisoning and the remaining seven died of unknown causes. Forty-nine (5.7%) of the birds in this study died as a result of collision with overhead cables. Road traffic accidents accounted for 18 (2.0%), disease 56 (6.5%) (bacterial and viral infections) and vandalism 21 (2.5%) (death due to stabbing, shooting by shotgun, cross bow, harpoon, air gun pellet and high velocity rifle bullet). A further 77 (8.9%) of deaths were of unknown causes and could not be placed in any of the above categories.

Chemical analyses of kidney, liver and bone showed that 74% (636) of the birds examined, had died of lead poisoning. These included the 562 mentioned above and 74 others with no lead shot in the gut. The levels of lead, expressed on a dry tissue weight basis, in a majority of the livers of the 636 poisoned swans (range 13-186 mg/kg^{-1}, mean 190 mg/kg^{-1}) exceeded the level (50 mg/kg^{-1}) widely recognised as indicative of lead poisoning. Similarly, the kidney levels (range

34-6330 mg/kg^{-1}, mean 620 mg/kg^{-1}) exceeded the limit (125 mg/kg^{-1}) usually taken to confirm lead poisoning in swans and other animals (Clarke & Clarke 1975, Simpson et al. 1979, Birkhead 1982). Sixty-eight birds with lower levels of lead in their tissues were also included in the lead-poisoned group, because the post-mortem and veterinary evidence were typical of lead-poisoned birds. The levels of lead in these 68 swans were (expressed as mg kg^{-1} dry wt): 13-38 (mean 32) in liver, 34-72 (mean 57) in kidney, and 40-92 (mean 66) in bone. Twelve of these 68 swans were the live birds that received a veterinary examination prior to death, and whose symptoms of lead intoxication have already been mentioned. Residue analysis also showed that significantly more females than males had died of lead poisoning (Table 1).

Of the swans that died with relatively low levels of lead in their tissues, 8 succumbed in the winter of 1981-82 and 4 in the winter of 1982-83, when temperatures of -17°C and -9.0°C respectively were recorded in the East Anglian region. These cold conditions could have exacerbated the hypothermia associated with lead poisoning in birds.

The pattern of lead deposition in the intoxicated and dead birds can provide evidence of the type of exposure that has taken place: a high bone and low soft tissue level indicates a slow chronic exposure, while

a low bone and high soft tissue level indicates a rapid acute exposure (Clarke & Clarke 1967). In all but 47 birds, kidney lead levels were higher than bone levels, indicating that death was due, in a majority of cases, to acute exposure.

Aerial Survey

On the aerial surveys, which were carried out each November during 1981-1984, the maximum number of swans counted at one time was 1232 (Table 2). This was in 1981, the first year of the survey. This figure also included the highest number (21%) of young (brown) birds. Thereafter numbers dropped. The lowest count (960) was in 1984, which also included the lowest number (135) of young birds.

Based on the total number of swans counted each year, the ratio of old to young birds changed from 1:0.21 in 1981 to 1:0.14 in 1984. Only 31 young (brown) birds (3.6% of the total) were received for post-mortem examination throughout this period.

Why the ratio of old to young birds found on aerial survey was not reflected in the bodies reported is difficult to explain. It is possible that the public were less likely to notice, or to report, brown birds than white ones.

Environmental lead levels

Soil and grass samples taken in the environmental survey (Table 4) show that lead levels were highest in samples taken in close proximity to the busy town centre, 460 mg kg^{-1} and 130 mg kg^{-1} respectively. One kilometer downstream from the city centre lead levels in the soil and vegetation had fallen to 92 mg kg^{-1} and 13 mg kg^{-1} respectively. Further downstream, lead levels slowly declined to 40 mg kg^{-1} for soil and 5.0 mg kg^{-1} for vegetation. The soil lead level near the city centre was lower than the average of 671 mg kg^{-1} in soils taken in 10 other British towns (Royal Commission on Environmental Pollution 1983).

Lead in the blood of the 18 swans sampled in the environmental survey ranged between 11 and 53 µg/100 ml blood (Table 4). Eight had blood lead values above 40 µg/100 ml. These levels compare well with the background blood lead levels of swans on the upper Thames and its tributaries. Above this figure in swans abnormal absorption of lead has probably occurred (see Birkhead 1983).

The seven samples of fish muscle analysed for lead were all below 1 mg kg^{-1} DW, in good agreement with previously published data from elsewhere (Mason et al. 1982). Levels of lead in the river water (0.5 mg kg^{-1}) were below that allowed by the local water authority,

Anglian Water, which has adopted a maximum permissible lead concentration of 0.5 mg dm^{-3} (Harrison and Laxen 1981).

The isotopic ratios of lead found in the live swan blood, bank soil and vegetation taken during the environmental survey were indistinguishable from one another, but were different from all the lead weights analysed in this way Table 5.

The main differences were as follows: (1) the Pb204 percentage abundance found in the lead weights differed from that found in the other samples, (2) the Pb206 percentage abundance found in the weights differed considerably from the blood and vegetation and also from the soil, although not to the same extent; (3) the Pb207 percentage abundance found in the weights differed from that found in the vegetation and soil and also from the blood, although not to the same extent; and (4) the Pb208 percentage abundance found in the weights differed from those found in the blood and vegetation, but the difference was more pronounced in the soil sample.

The main conclusion to be derived from these comparisons was that lead in soil, water, vegetation and blood from healthy swans was derived largely from the same sources, whereas lead in fishing weights differed in composition.

Grit Status

For this exercise, 100 birds were used, 50 control and 50 lead-poisoned.

Only 17% by weight of the grit from the control group and 16% of the grit from the lead-poisoned group was retained by the 1 mm sieve. These figures are in general agreement with the work of Owen and Cadbury (1975) who found that 90% of the gizzard grit of mute swans was less than 2 mm in diameter. A collection of new fishing weights was also sized in this way. Fishing weights of size 12 were retained by a 1 mm sieve. Sizes 3, 7 and 8 by a 2 mm sieve, BB by a 3 mm and AAA by a 4 mm sieve. Thirty-eight percent of the lead weights and lead fragments removed from the lead poisoned swans were retained by the coarsest sieve (4 mm) and 54% by the 2 mm sieve. Of all the fragments which passed through the 2 mm sieve, 6% were retained by the 1 mm sieve and showed considerable erosion. Only 2% passed through the finest sieve. Results from this experiment show that swans preferentially ingest fishing weights of a larger size than the normal gizzard grit.

The total weight of gizzard grit was not significantly different between the two groups, lead poisoned mean 81.1 g (range 56.1-103 g) and control group mean 76.4 (range 48.3-126 g) ($t = 0.16$, DF 98, $P = 0.8672$).

Carcass Recovery Experiment

The carcasses of 23 birds were taken back and left where they were originally found. Of these, 4 were notified by members of the public and a fifth bird was collected from the local police having been taken to them by a member of the public. We were informed by the Peterborough Development Corporation that they had disposed of a further 3 marked carcasses. Within 12 days, 16 birds had disappeared including 2 of the reported birds. Four bodies remained unreported for 20 days after which time they were removed and the study terminated. Out of the 23 birds put out fifteen were never reported.

DISCUSSION

The large number of swans reported dead and confirmed as lead poisoned in this study is consistent with previous findings from other areas (Simpson et al. 1979; NCC 1981; Birkhead 1982; Sears 1988). The number of female swans dying of lead poisoning, which was not found in previous studies, is difficult to explain. Breeding female swans need calcium for egg production, and this can be furnished either by mobilising stored calcium from the skeleton - in chickens up to 40% of the stored calcium can be used in this way (Sturkie 1954) - or by an increased absorption from food in the alimentary tract (Barltrop & Khoo 1976). Because the metabolism of lead follows closely that of calcium,

a high demand for calcium from either source could yield a high availability of lead to the soft tissues. Female ducks in breeding condition are known to accumulate more lead in their soft tissues than do males (Finley et al. 1976; Finley & Dieter 1978).

The levels of lead found in swans' blood could have originated from many sources: industrial pollution, ingestion of shotgun pellets, fishing weights or food contaminated with airborne lead from motor vehicle exhausts. High blood lead levels in London pigeons (Columba livia) were attributed to food contaminated by roadside dust (Hutton 1980). The increase in swan blood lead values with proximity to London has been attributed to the increased availability of fishing weights (Birkhead 1982). The lead isotope ratio analysis conducted on specimens taken during my survey show that background levels of lead found in the live swan blood most probably originated from one source, motor vehicle emissions (Sears 1988).

During the carcass recovery experiment 8 (35%) bodies were reported, but only 5 (22%) of the birds would have been made available for post-mortem examination and chemical analysis; of these 3 (60%) had already been shown to have died of lead poisoning. The surprising fact was that 16 (69%) of the bodies disappeared without trace possibly having been taken by foxes or other scavengers. Of these, 7 (30%)

disappeared in the first 48 hours. Four (17%) remained present but unreported until the end of the study. The recovery rate partly reflected public awareness of the need to report dead or dying swans and would have been unusually high during the study period. At that time newspapers, radio and television stations, both local and national, featured the plight of lead-poisoned swans. Increased public reports of dead or unhealthy swans invariably followed this media exposure, but still 15 (65%) of the carcasses were never reported. Less than 2% (15) of the 860 swan bodies examined in this survey were reported by the police, water bailiffs or lock keepers.

Interestingly, the proportion of carcasses reported in this study (35%) was very similar to the proportion (33%) found by Perrins (1981) from ring recoveries in southern England.

Although there was no way to check the accuracy of the aerial counts, they were carried out within one day at the same time of the year for four consecutive years, and therefore must have given some indication of the number of swans in a major proportion of the study area. They indicated that during the years 1981-1984 numbers declined. The average number of swans found dead each year, throughout the study area amounted to 200. Based on the results of the carcass recovery experiment, where 35% of the bodies were re-notified, the total number

of swan deaths per year could be estimated at around 570, of which around 420 could have died of lead poisoning and around 150 of other causes.

These figures show that the population in the study undercounted the number of birds present, that the carcass recovery experiment underestimated the proportion of deaths reported, or that the population was partially maintained by net immigration of birds from outside the study area.

Either way, it is not surprising that deaths due to lead poisoning were associated with population decline in this area, as in some other parts of Britain.

The results from the grit study pose the question whether swans eat lead weights by accident or choice. The evidence suggests they do so by choice. Throughout the study area, samples of sediment and soil contained grit ranging in size from less than 1 mm to 5 mm and over. The larger grit and stones were rarely found in gizzard contents. There was no significant difference in total grit weight or size between the two groups of swans tested, indicating that swans are able to select the various grit sizes they require and that the lead poisoned group were not grit deficient. However, in the lead-poisoned

group, lead recovered from the gizzards was nearly always larger than the largest grit. These findings tend to mitigate against the idea that a majority of weights are accidentally ingested along with grit.

Some weights may have been eaten along with discarded bait or accidentally ingested when swans tried to free themselves from line entanglement (Sears 1988).

Birkhead (1982) and Sears (1988) in the Thames catchment showed a sharp decline in swan lead poisoning incidents during March-June (which includes the closed fishing season). In both studies there was also a rapid increase in the number of lead-poisoned swans reported at the start of the fishing season. The combined post-mortem results from the period of my study in East Anglia showed that disproportionately more swans died of lead poisoning during the period March-June, which includes the closed fishing season, and that relatively fewer died from other causes during July-October. Swans can take up to 3 weeks to die from lead intoxication after ingesting fishing weights, so this fact must be taken into account when interpreting the closed season data.

As a member of the Nature Conservancy Councils' working group on lead poisoning in swans, my results along with those of others, helped to provide sufficient evidence to force legislative action. In 1987 new

laws were introduced banning the sale or importation of lead weights for fishing in the range 0.06 g up to 28.36 g to help protect the indigenous swan population. A total ban on the use of lead weights in this range was adopted by the ten English and Welsh Water Authorities in 1987. Since 1985 alternative fishing weights have been readily available and their forced adoption in 1987 has lead to a marked decline in the incidence of lead poisoning in many areas (Sears 1989, Hunt pers. comm.), thus confirming the mortality caused by the widespread use of lead weights. Similar declines in waterfowl lead poisoning incidents were noted in the United States following the banning of lead gunshot. In Missouri, following the introduction of steel shot, lead poisoning incidents declined (Humburg and Babcock 1982). And in Illinois, Welch (1979) estimates that almost 15,000 mallard were saved because they ingested steel rather than lead shot.

Sears (1988) predicted that, if lead fishing weights were no longer used, incidents of lead poisoning should rapidly decline. However, drought may make available fishing weights that were previously inaccessible due to water depth, a situation previously recorded with gun shot (Anderson 1975).

REFERENCES

Anderson, W.L. (1975). Lead poisoning in waterfowl at Rice Lake. Ill. J. Wildl. Mgmt., **39**, 264-

Barltrop, D. & Khoo, H.E. (1976). The influence of dietary minerals and fat on the absorption of lead. Sci. Total Environ., **6**, 265-273.

Beer, J.V. & Stanley, P. (1964). Lead poisoning in the Slimbridge Wildfowl collection. Rep. Wildfowl Trust, 16th, 1963-64, 30-34.

Belrose, F.C. (1959). Lead poisoning as a mortality factor in waterfowl populations. Bull. Ill. St. nat. Hist. Surv., **27**, 235-288.

Birkhead, M.E. (1982). Causes of mortality in the mute swan on the River Thames. J. Zool., **198**, 15-25.

Birkhead, M.E. (1983). Lead levels in the blood of mute swans Cygnus olor on the River Thames. J. Zool. Lond., **199**, 59-73.

Campbell, M.J. & Delves, H.T. (1989). Accurate and precise determineation of lead isotope ratios in clinical and environmental samples using inductively coupled plasma source mass spectrometry. J. Anal. Atom. Spec. **4**, 235-236.

Clarke, E.G.C. & Clarke, M.L. (1967). Garner's Veterinary Toxicology. 3rd ed. New York: Williams & Wilkins.

Clarke, E.G.C. & Clarke, M.L. (1975). Veterinary Toxicology. 3rd ed. London: Bailliere.

Clausen, A.G., Dalsgaard, H. & Wolstrup, C. (1975). Udbrid af blyforgiftning blandt danske knopsvaner (Cygnus olor). Dansk. Vet. Tidsskr., **21**, 843-847.

Control of Pollution (Anglers Lead Weights) Regulations 1986.

Del Bono, G. (1970). Il saturnismo degli uccelli acquatici. Annali Fac. Med. vet. Univ. Pisa, **23**, 102-151.

Finley, M.T. & Dieter, M.P. (1978). Influence of laying on lead accumulation in bone of mallard ducks. J. Toxicol. Environ. Health, **4**, 123-129.

Finley, M.T., Dieter, M.P. & Locke, L.N. (1976). Lead in tissues of mallard ducks dosed with two types of lead shot. Bull. Environ. Contam. & Toxicol., **16**, 261-269.

French, M.C. (1982). Lead poisoning in Bewick swans. BTO News, No.121, 1.

Harrison, R.M. & Laxen, D.P.H. (1981). Lead Pollution Causes and Control, Chapman & Hall. ISBN 0-412-16360-8.

Humburg, D.D. & Babcock, K.M. (1982). Lead poisoning and lead/steel shot: Missouri studies and a historical perspective. Miss. Dept. Cons. Tech. Rep. No. 10.

Hunt, A.E. (1977). Lead poisoning in swans. BTO News, **90**: 1-2.

Hutton, M. (1980). Metal contamination of feral pigeons. Columba livia from the London area: Part 2 Biological effects of lead exposure. Envir. Pollut., **22(A)**, 281-293.

Locke, L.N., Kerr, S.M. & Zoromski, D. (1982). Lead poisoning in common loons (Gavia immer). Avian Dis., **26**, 392-396.

Mason, C.F., Macdonald, S.M. & Aspden, V.J. (1982). Metals in Freshwater Fishes in the United Kingdom 1980-81. A report to The Vincent Wildlife Trust, Baltic Exchange Buildings, 21 Bury Street, London.

Nature Conservancy Council (1981). Lead poisoning in swans. London: NCC.

Owen, M. & Cadbury, C.J. (1975). The ecology and mortality of swans on the Ouse Washes, England. Wildfowl 31-42.

Sears, J. (1988). Regional and seasonal variations in lead poisoning in the mute swan Cygnus olor in relation to the distribution of lead and lead weights, in the Thames Area, England. Biol. Cons., **46**, 115-134.

Sears, J. (1989). A review of lead poisoning among the River Thames mute swan Cygnus olor population. Wildfowl **40**, 151-152.

Shields, J.B., Mitchell, H.H. & Ruth, W.A. (1978). The metabolism and retention of lead in growing and adult rats. J. Ind. Hyg. Toxicol., **21(1)**, 7-23.

Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of mute swans. Environ. Pollut., **18**, 187-202.

Sturkie, P.D. (1954). Avian Physiol. New York: Comstock Publishing Associates.

Perrins, C.M. (1981). Mortality of mute swans Unpub. Rep. to the working group on lead poisoning in mute swans.

TABLE 1
Sex distribution of birds dying of lead poisoning and other causes.

	Lead poisoned	Other than lead poisoned
Males	182	93
Females	454	131

$X^2 = 12.84$, d.f.1, $P < 0.001$

TABLE 2

Data on the numbers of swans counted on the River Ouse, Nene and Welland and adjacent gravel pits etc, during November 1981-1984.

Number of young (brown birds) included in the total is shown in brackets

	Ouse	Nene	Welland	Total
1981	592 (126)	306 (81)	334 (54)	1232 (261)
1982	565 (104)	274 (61)	303 (45)	1142 (210)
1983	521 (32)	296 (92)	292 (34)	1109 (158)
1984	481 (77)	230 (40)	244 (18)	960 (135)

TABLE 3
Seasonal distribution of deaths from different causes during October 1981-September 1985*

	November-February	March-June	July-October
Lead-poisoned	206	245	156
Other than lead-poisoned	51	57	56

$X^2 = 12.52$, 2 d.f., $P < 0.05$

* Stopped at this date in order to give four complete years of data and an unbiased seasonal pattern.

TABLE 4

Lead levels on various samples taken during the environmental survey.

	Total Lead ppm D/wt						
	Soil	Land Vegetation	Water Vegetation	Fish	Water ppm	Swan Blood Lead	
City Centre	460	130	1	1	1	16,30,37,43,52	5 swans sampled
1.2 km from city centre	90	12	1	1	1	26,35,44	3 swans sampled
3.2 km from city centre	58	8	1	1	1	38,46,50,53	4 swans sampled
4.8 km from city centre	54	5	1	1	1	11,19,37,44	4 swans sampled
8.0 km from city centre	40	5	1	1	1	23,48	2 swans sampled

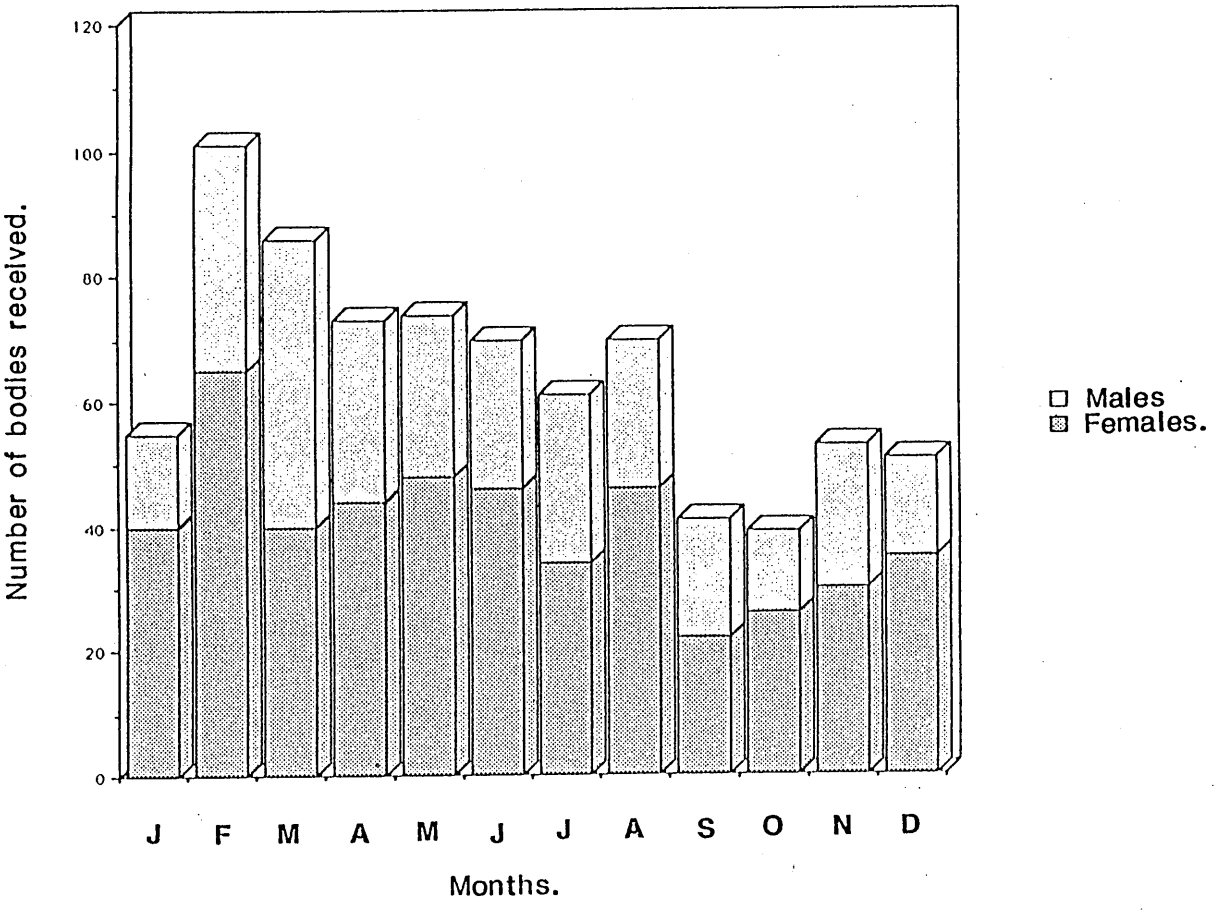
Table 5.

Lead values and percentage abundance of four lead isotopes in samples of lead weights, swan blood, soil and vegetation collected during the environmental survey.

		Lead values expressed as µg/100 ml blood, soil and vegetation as ppm D/wt.	Pb204	Pb206	Pb207	Pb208
Lead weights	1		1.403	24.376	21.770	52.451
	2		1.400	24.224	21.985	52.391
	3		1.401	24.367	21.781	52.451
Mean			1.401	24.322	21.845	52.431
Whole blood	1	43	1.410	24.046	22.434	52.110
	2	44	1.411	24.048	22.430	52.111
	3	50	1.408	24.044	22.439	52.109
Mean			1.409	24.046	22.437	52.110
Vegetation	1	130	1.411	24.057	22.422	52.110
	2	12	1.407	24.055	22.421	52.117
	3	8	1.411	24.052	22.422	52.115
Mean			1.409	24.054	22.421	52.114
Soil	1	460	1.411	24.100	22.399	52.090
	2	90	1.410	24.047	22.431	52.112
	3	58	1.408	24.044	22.440	52.108
Mean			1.409	24.063	22.423	52.103

Sampling points 1 = City Centre
2 = 1.2 km from City Centre
3 = 3.2 km from City Centre

One swan blood from each of the 3 sampling points was selected for isotope analysis.



The total number of swan bodies received each month between October 1981 and September 1985.

Figure 1.

Chapter 3

Seasonal Variation in Tissue Lead and Zinc Values in the feral Pigeon (*Columba livia*) following a period of Lead Dosing

INTRODUCTION

Although much of the literature on lead poisoning in swans and other waterfowl is based on tissue analysis (see Chapter 2 for review), a few intensive blood lead monitoring programs on swans have been carried out (Birkhead 1983, Sears 1988, O'Halloran 1989). It is generally accepted that lead levels of 50 mg/kg and 125 mg/kg on a dry weight basis respectively in the liver and kidney of swans are indicative of lead poisoning (Birkhead 1982, Simpson et al. 1979). These levels are applied to birds of most age groups, both sexes and irrespective of season or body condition. Seasonal variations in both toxic and essential metal content of avian livers are known to occur (Osborn 1979, Hagen et al. 1976 & Haarakangas et al. 1974). Seasonal changes in the body condition of various birds, as reflected in lipid and protein reserves, are also well documented (Ward 1977, Ward and Jones 1977). Hence, seasonal changes in the metal content of avian liver could be due either to seasonal availability or absorption of a particular metal or to a shift of that particular metal from one body compartment to another associated with body condition. The existence of such seasonal cycles poses the question whether birds should be

assigned to poisoned or non-poisoned categories on residue levels alone, or on a combination of measurements which include seasonal variations. The objectives of this study were:

(1) to investigate trends in liver lead levels after a period of dosing had ceased;
(2) to help determine the role which zinc plays in lead intoxication, especially during the moult; and
(3) to determine tissue lipid and protein levels as an indicator of physiological condition.

MATERIALS AND METHODS

Some studies have shown sex differences in liver metal levels (Evans and Moon 1981, Hoffman and Curnow 1979, Parslow et al. 1982), while other studies have not (Hulse et al. 1980, Flemming 1981). With this possibility in mind this program of work was confined to male pigeons (Columba livia). Forty birds, all bearing identity rings, were each given daily doses of 2.5 mg of lead nitrate incorporated in a gelatin capsule for 5 consecutive days, to make a total dose of 12.5 mg lead nitrate. The birds were housed in groups of 20 in two adjacent flight pens 4 x 2 x 2 m high. They were allowed free access to food which

consisted of wheat, maple peas, beans and maize, and to oyster-shell grit and water. Two 14 litre plastic bowls filled with water were provided for bathing and this water was changed daily.

A control group of 16 male birds was housed in a separate pen and similarly treated but given no lead nitrate. During the study two control birds were killed by vermin. All the test birds survived until sampled. Twenty four hours after the final dose of lead nitrate, three dosed birds and one control bird were selected at random and killed by cervical dislocation. Each bird was dissected immediately, and samples of liver and blood were taken for chemical analysis. Blood samples were also taken from the remaining live birds. This process was repeated at the end of each calendar month each time selecting three dosed birds and one control bird at random. During the first month, blood samples were also taken 7 and 14 days after dosing had ceased.

The liver samples removed at post-mortem from both the dosed and control birds were analysed for lead and zinc, water and fat content. Blood samples were analysed for lead only.

Determination of fat content of liver

A sample of fresh liver was weighed into a beaker and then ground with fine sand and anhydrous sodium sulphate. The mass was then extracted

with 5 ml quantities of a mixture of acetone and hexane. The solvent mixture was decanted off into a measuring cylinder and the process repeated until a volume of 50 ml had been obtained. The cylinder contents were then shaken and allowed to stand for 24 hours to allow any fine sand or sodium sulphate debris to settle out. Fat levels were determined by decanting 25 ml of the clear extract into a weighed vial and allowing the solvent to evaporate off before reweighing the vial. The increase in weight X2 was taken as the fat content.

RESULTS

Diet Lead Levels

Bathing and drinking water was taken from the domestic supply and lead levels were below 1 ppm.

Water in the 14 litre bathing bowls was also regularly analysed to establish whether faecal contamination was causing any elevation of lead levels. Together with the wheat, maple peas, beans and maize used for food, residues of lead were always below 1 ppm.

Blood lead levels

Initially, the median lead values in blood (Figure 1) were very high, at 228 µg/100 ml. Median levels then halved in approximately seven

days to a value of 106 µg/100 ml and then halved again in a further 24 days (31 days after dosing) to 56 µg/100 ml. All the values remained above the 40 µg/100 ml blood established as a maximum acceptable level (M.A.L.) for lead in the blood of swans (Birkhead 1983). The highest blood lead value in the control birds was 14 µg/100 ml, and the remainder were between 5 and 9 µg/100 ml, with an overall mean of 7 µg/100 ml blood.

Liver fat levels

Mean fat levels in liver steadily declined for seven consecutive months (January to August) with a slight but insignificant peak occurring in June. Levels in August (38 mg/g) had fallen to approximately one third of their January value (96 mg/g) (Fig. 2). During the following four months (September to December) fat levels increased, gradually returning to within 10% of the original January value.

Liver lead levels

The overall liver lead levels gradually declined during the study (Fig. 3) from 22 mg kg^{-1} to 7.1 mg kg^{-1} (expressed on a dry weight basis). However, a significant peak of 22 mg kg^{-1} occurred in August, coinciding with the lowest mean fat level (Fig. 2). Liver lead levels remained below the value 50 mg kg^{-1}/DW usually taken as indicative of lead poisoning in birds and mammals (Clarke and Clarke 1975, Simpson et al. 1979). Whole organ load of lead also showed the same pattern (Fig. 4).

Liver protein levels

Crude total protein levels in liver were calculated by subtracting the lipid levels from the dry weight and then adjusting for total organ size. Contrary to expectation, protein levels remained remarkably constant during the experiment (Fig. 2) and did not show the marked seasonal trend previously found in starlings (Sturnus vulgaris) (Osborn 1979, Ward 1977).

Liver zinc levels

The dry weight results (Fig. 5) showed a peak in zinc concentration in June (222 mg kg^{-1}) just prior to the moult, with lower levels occurring during the moult in July, August and September (148, 146 and 126 mg kg^{-1} respectively). These results are similar to those earlier demonstrated by Osborn (1979) and Haarakangas et al. (1974). The low zinc levels during July-September also correspond to the peak in lead residues during those months.

DISCUSSION

Whether expressed as whole organ load or concentration, the peak in liver lead levels, which occurred in August, was significant compared with values found in June. The possibility exists that the method of

expressing protein is incorrect and a more accurate method (Lowry et al. 1951) should have been used. However, it is difficult to see what could have influenced the protein levels determined by my method of calculation. In his study, Osborn (1979) suggests that the observed increase in cadmium could be indicative of an increase in the amount of a particular protein (namely metallothionein) rather than an overall increase in protein levels. In rats an increase in liver mass accompanied by an increase in total hepatic protein occurred 48 hours after receiving a single dose of lead nitrate (Ledda et al. 1982). In birds this protein may increase just before the moult in order to retain zinc which is known to be in especially high demand during feather formation (see Underwood 1977).

The findings in this experiment go some way to support this theory in that zinc is accumulated by the liver and maybe other tissues for possible use during the moulting process, without a significant change in protein concentration. The possibility further exists that the high demand for zinc during the moult (Underwood 1977) may cause an overall zinc deficiency, and the elevated lead values in August may then be due to a change in the nutritional status of the animal associated with the moult. In rats the levels of dietary intake of, amongst other things, zinc are known to affect the toxicity or absorption of lead and cadmium (Petering 1978), so zinc could be exerting a degree of protection against lead intoxication (Chapter 10, Cerklewski and Forbes 1976).

As the zinc stored in the liver increased during June, in my birds the total lead load decreased, increasing again during July, August and September as the zinc was utilised in feather production.

Porphyrin biochemstry is affected by lead intoxication resulting in excess excretion of coproporphyrin, protoporphyrin (Waldron and Stöfen 1974) and uroporphyrin (Bashour 1954). In humans, increased excretion of uroporphyrin has been shown to cause excessive loss of zinc by the chelating action of the former (Pfeiffer 1987). This situation may also affect the zinc balance in a lead-intoxicated bird and further contribute to metal instability.

The gradual decline in blood lead values shown throughout the study, not withstanding the peaking of liver lead concentrations during August, support the previously published work on swans (Birkhead 1983, Sears 1988). In their studies, the fluctuating availability of lead, in the form of fishing weights, caused seasonal changes in blood lead values. My present study further shows that a change in the physiological state of the animal, associated with moult together with elevated liver lead and zinc concentrations, had no detectable effect on blood lead concentrations. This point is also investigated in Chapter 4. This experiment has also shown that rapidly falling blood lead values are an obvious reflection of recent exposure, ie within the

last 30 days. Starvation, stress, moult, reproduction and general ill health have also been shown to affect body condition and physiological state in one way or another (Sears 1988, O'Halloran 1989).

This study clearly supports the idea of Osborn (1979), that the biochemical composition of tissues and the physiological state of the animal at the time of collection or death, must be taken into account when assessing the degree of contamination or the effect that any particular residue may be having. Although more time-consuming, analysis of whole bodies, together with fat and protein analysis, is probably better for producing evidence of residue effect than single tissue analysis. In this study the birds were well fed and at post-mortem showed considerable fat reserves. This situation may have had a buffering effect on changes in body composition. Birds in "field condition" may show a more marked fluctuation in compartmental shift of metals, especially during stress.

This study also shows that a degree of caution must be exercised when comparing lead levels in birds sampled at different times of the year.

REFERENCES

Bashour, F.A. (1954). Urinary Uroporphyrin, Porphobilinogen and Coproporphyrin excretion in lead-exposed workers. J. Lab and Clin. Med., **44**, 764-765.

Birkhead, M. (1982). Causes of mortality in Mute Swans Cygnus olor on the River Thames. J. Zool., Lond., **198**, 15-25.

Birkhead, M. (1983). Lead levels in the blood of Mute Swans Cygnus olor on the River Thames. J. Zool., Lond., **199**, 59-73.

Cerklewski, F.L. & Forbes, R.M. (1976). Influence of dietary zinc on lead toxicity in the rat. J. Nutr., **106**: 689.

Clarke, E.G. & Clarke, M.L. (1975). Veterinary Toxicology, 3rd ed. London, Baillere.

Evans, P.R. & Moon, S.J. (1981). Heavy metals in shorebirds and their prey in north-east England. In Heavy metals in northern England, environmental and biological aspects ed. P.J. Say & P.A. Whitton, 181-90. Univ. Durham.

Flemming, W.T. (1981). Environmental metal residues in tissues of canvas backs. J. Wildl. Mgmt., **45**, 508-511.

Haarakangas, H., Hyvarinen, H. & Ojanen, M. (1974). Seasonal variations and the effects of nesting and moulting on liver mineral content in the house sparrow (Passer domesticus L.). Comp. Biochem. Physiol., **47A**, 153-63.

Hagen, J., Hagen, A., Østbye, E. & Skar, H-J. (1976). Some chemical elements in the body of the Meadow Pipit. Norwegian J. Zoology, **24**, 279-289.

Hoffman, R.D. & Curnow, R.D. (1979). Mercury in herons, egrets and their foods. J. Wildl. Mgmt., **43**, 85-93.

Hulse, M., Mahoney, J.S., Schroder, J.D., Hacker, C.S. & Pier, S.M. (1980). Environmentally acquired lead, cadmium and in Cattle Egret, Bubulcus ibis, and the Laughing Gull, Larus atricilla. Arch. Environ. Contam. Toxicol., 9, 69-78.

Leeda, G.M., Columbano, A., Perra, T. & Pani, P. (1982). Stimulation of rat liver growth by a single administration of lead nitrate. Toxicol. Appl. Pharmacol. **65**, 478-480.

Lowry, O.H., Rosenbrough, A.L., Farr, A.L. & Randall, R.J. (1951). Protein measurements with the Folin-phenol reagent. J. Biol. Chem., **193**, 265-72.

Murton, R.K. & Westwood, N.J. (1977). Avian Breeding Cycles. Oxford Univ. Press. ISBN 0-19-857-357-X.

O'Halloran, J. & Myers, A.A. (1989). Some sublethal effects of lead on Mute Swan Cygnus olor. J. Zool. Lond., **218**, 627-632.

Osborn, D. (1979). Seasonal changes in the fat, protein and metal content of the liver of the starling Sturnus vulgaris. Environ. Pollut., **19**, 145-155.

Parslow, J.L.F., Thomas, G.F. & Williams, T.D. (1982). Heavy metals in the livers of waterfowl from the Ouse Washes, England. Environ. Pollut., Ser. A, **29**, 217-327.

Petering, H.G. (1978). Some observations on the Interaction of zinc, copper and iron metabolism in lead and cadmium toxicity. Environ. Health Perspect., **25**, 141-145.

Pfeiffer, C.C. (1987). Mental Illness and Schizophrenia. The Nutrition Connection. Thorsons, England. ISBN 0-7225-1465-4.

Sears, J. (1988). Assessment of body condition in live birds; measurements of protein and fat reserves in the mute swan, Cygnus olor. J. Zool., Lond., **216**, 295-308.

Sears, J. (1988). Regional and seasonal variations in lead poisoning in the Mute Swan Cygnus olor in relation to the distribution of lead and lead weights in the Thames Area, England. Biological Conservation **46**, 115-134.

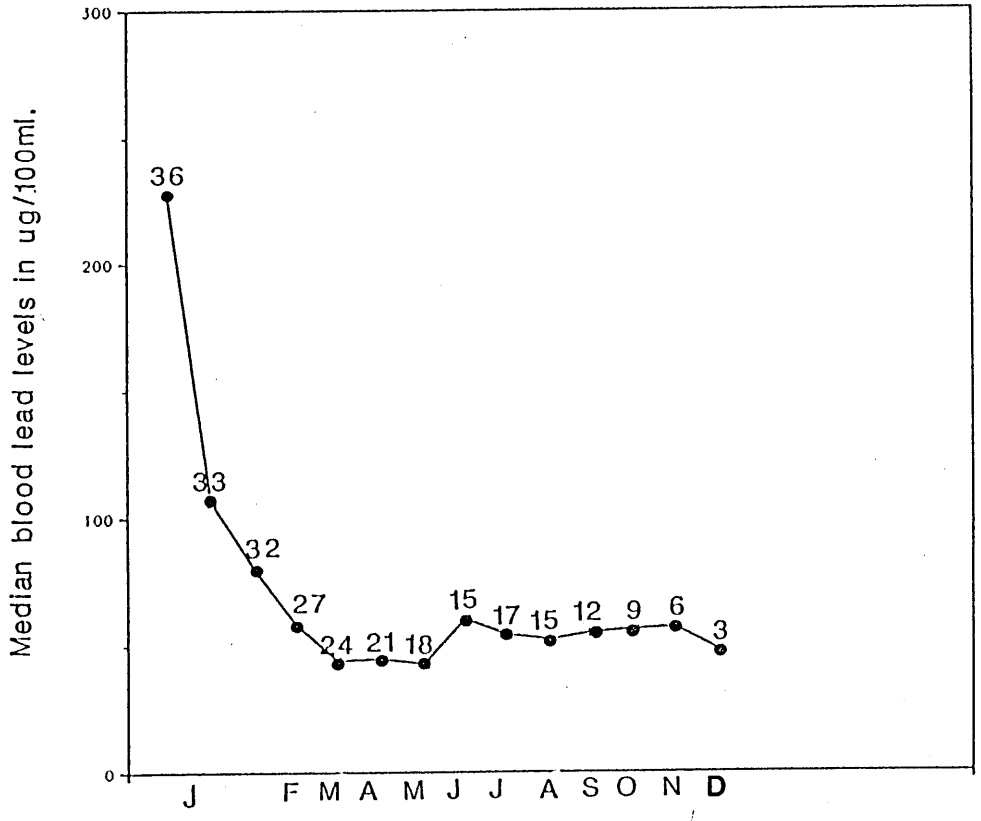
Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of Mute Swans. Environ. Pollut. **18**: 187-202.

Underwood (1977). Trace elements in human and animal nutrition. Academic Press ISBN 0-12-709065-7.

Waldron, H.A. & Stofen, D. (1974). Sub-clinical Lead Poisoning. Academic Press, London, New York. ISBN 0-12-671650-1.

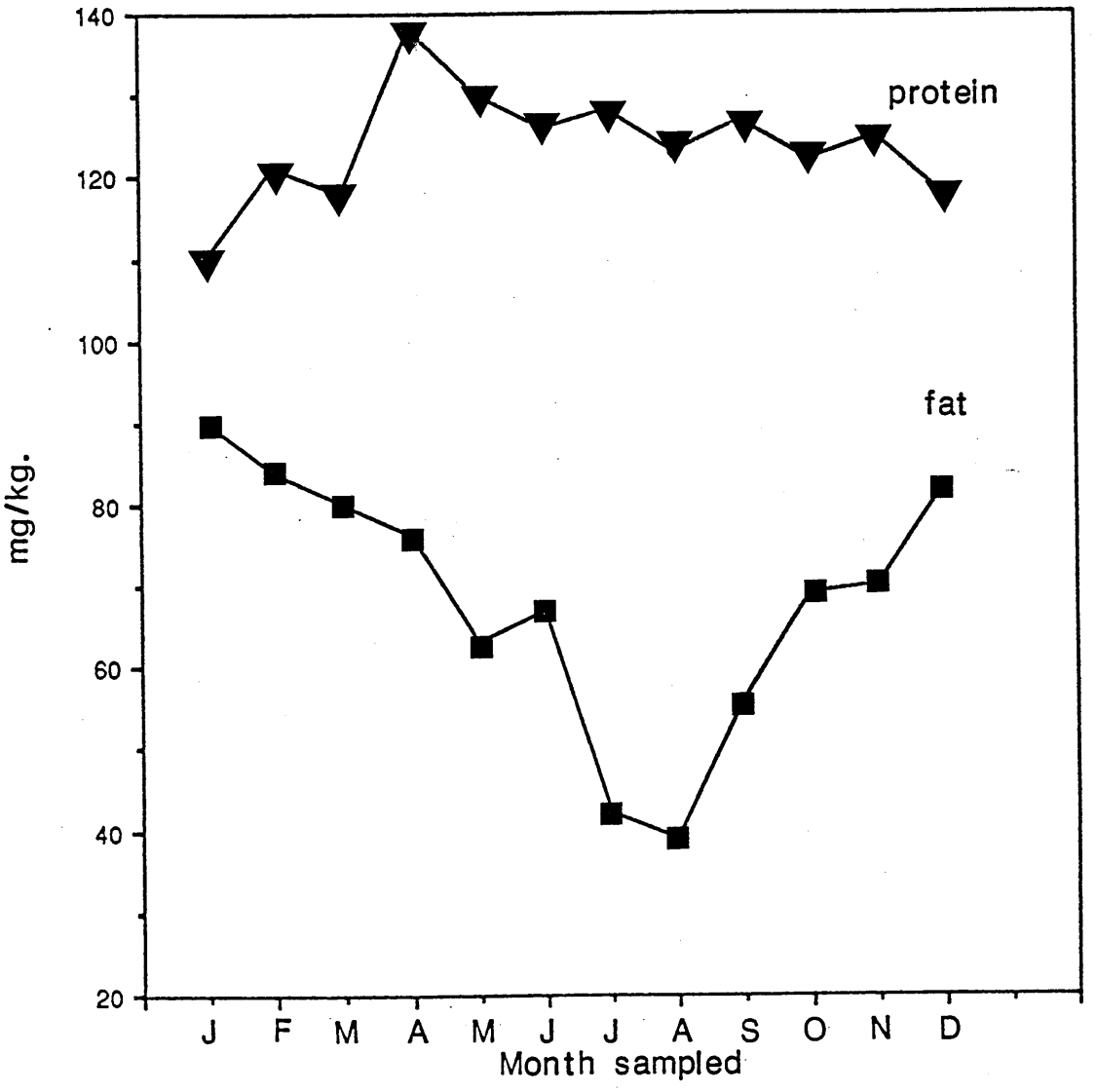
Ward, P. (1977). Report of the Institute of Terrestrial Ecology, Natural Environment Research Council, p.54-56.

Ward, P. & Jones, P.J. (1977). Pre-migratory fattening in three races of the Red-billed quelea Quelea quelea (Aves: Ploceidae), an intratropical migrant. J. Zool., Lond. **181**, 43-56.



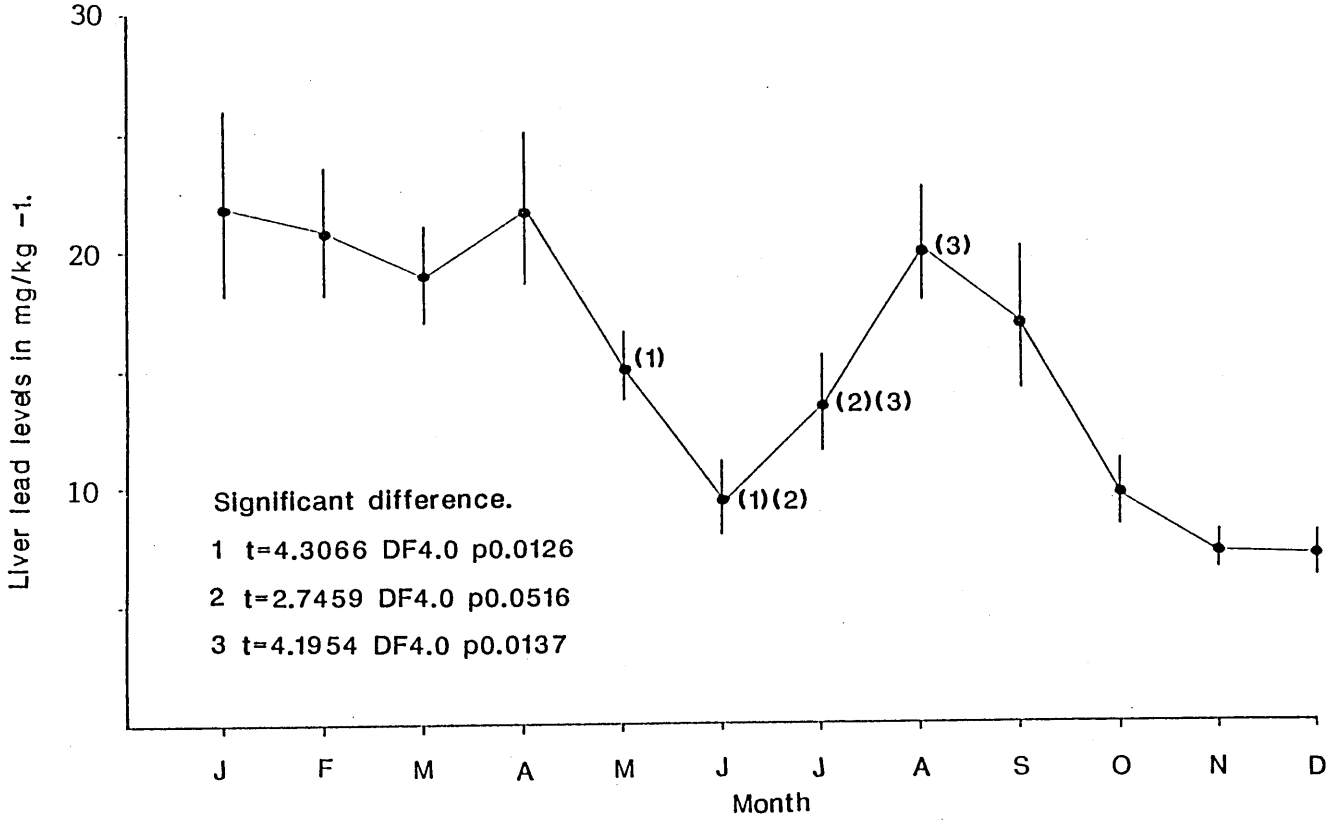
Monthly median blood lead levels.Sample size given above each point.

Figure 1.



Seasonal changes in mean fresh liver tissue concentration of protein and fat.

Figure 2.



Seasonal changes in liver dry weight lead concentration .

Symbols represent ±1SE.

Figure 3.

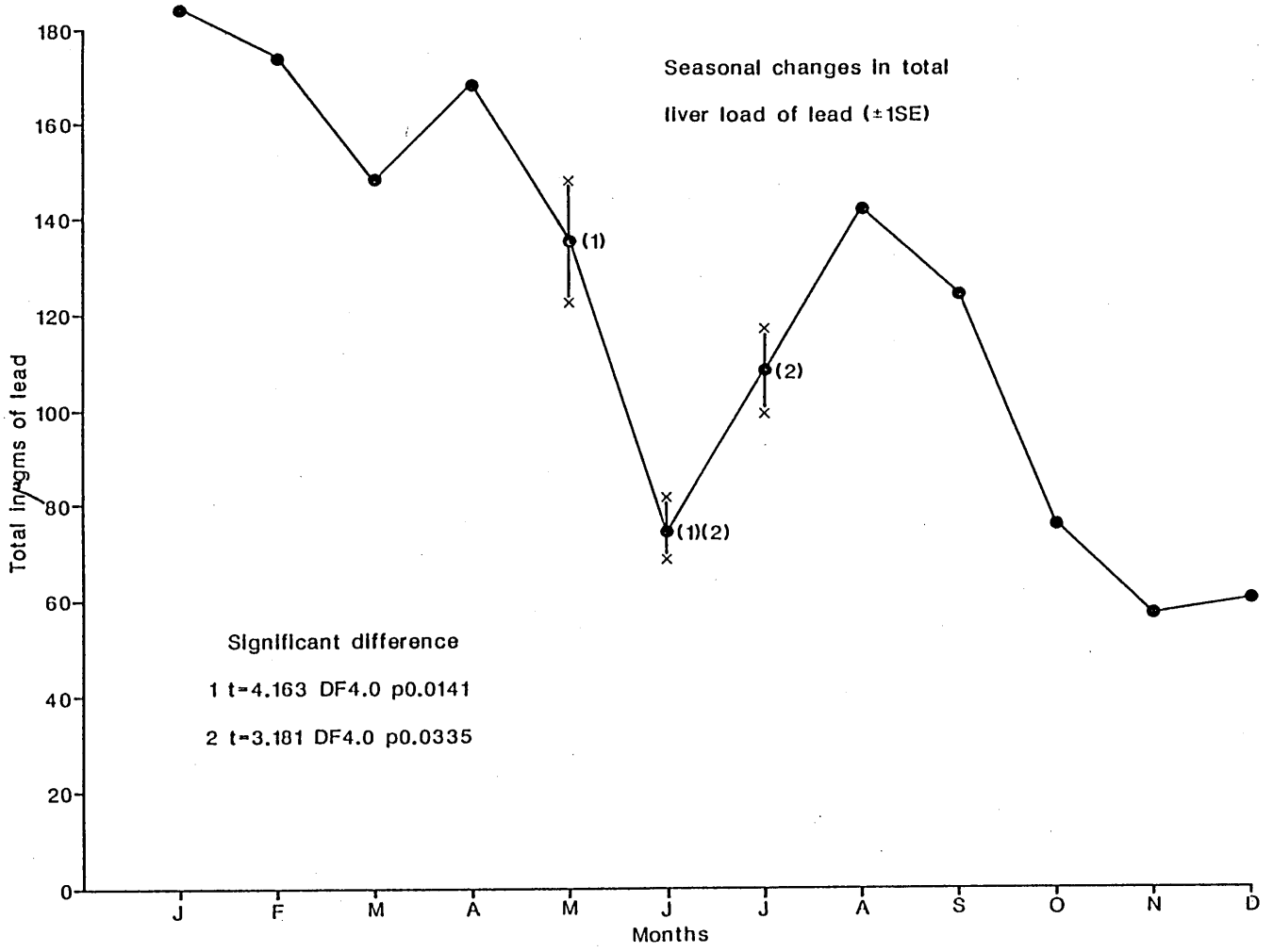

Seasonal changes in total
liver load of lead (±1SE)
Total in gms of lead
180
160
140
120
100
80
60
40
20
0
(1)
(2)
(1)(2)
Significant difference
1 t=4.163 DF4.0 p0.0141
2 t=3.181 DF4.0 p0.0335
J
F
M
A
M
J
J
A
S
O
N
D
Months

Fig 4

Seasonal changes in liver dry weight zinc concentration symbols represent ±1SE

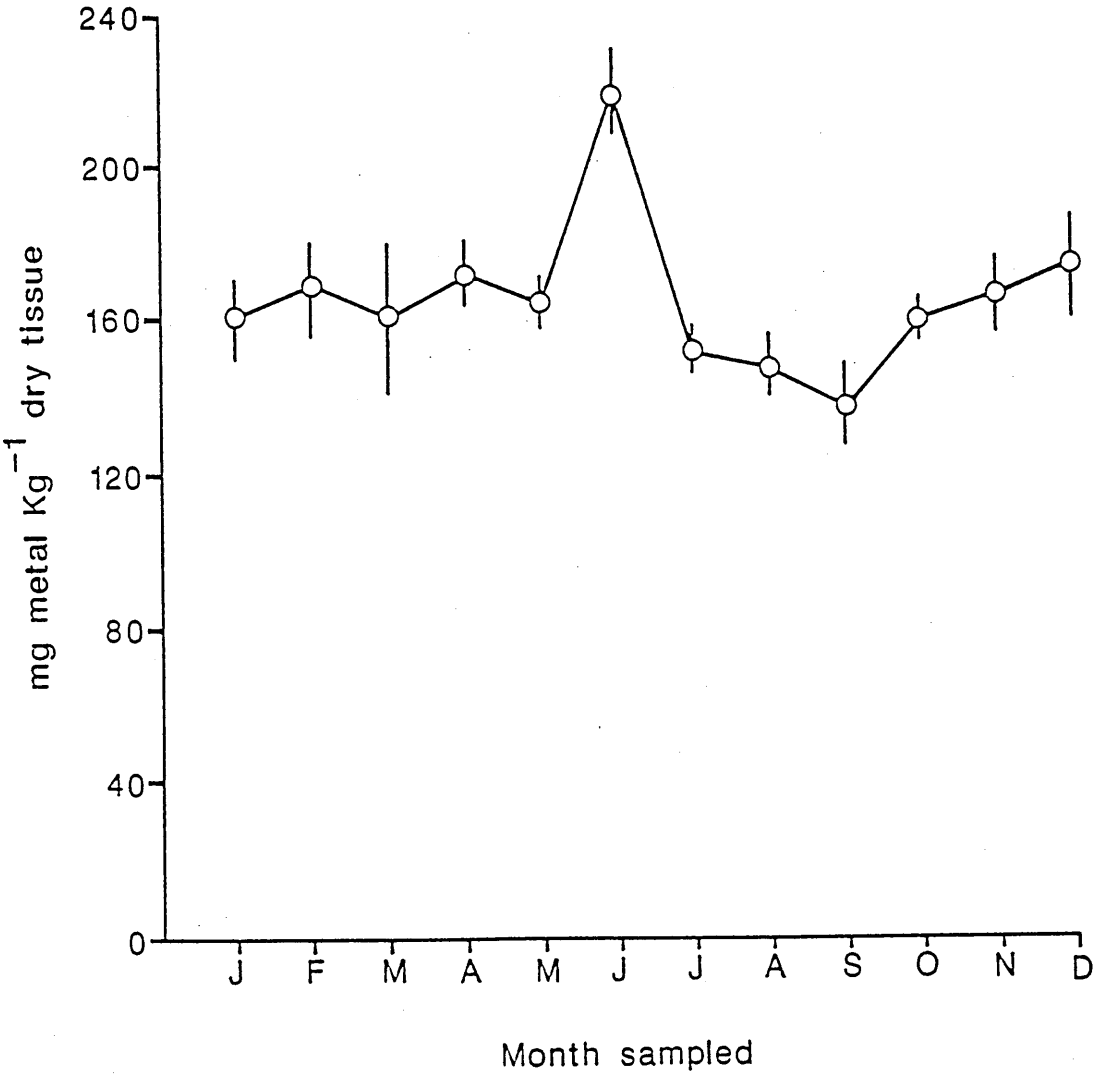


Fig 5

Chapter 4

Blood Lead Values - Effect of Calcium Metabolism during Egg Shell Formation in Pigeons (*Columba livia*)

INTRODUCTION

The transfer of lead from gut to body tissues is carried out by the blood, where it is bound to the red blood cells or the plasma proteins, but a small amount is thought to be in the free ionised form. In vitro studies have shown that, at low dose, 95% of the lead is bound to the red blood cells (RBCs) (Clarkson & Kench 1958), and the method of attachment has been established (Clarkson & Kench 1958, Birkhead 1982).

Much of the body lead is transferred by the RBCs to the skeletal bone where up to 90% of the total body burden can be found (Harrison & Laxen 1981). Lead is able to replace calcium in the bone, although only about 0.65% of the bone calcium is exchangeable (Rowland 1966). The reasons why lead and calcium can interchange have yet to be elucidated but, in man, parathyroid hormone injections can enhance the removal of lead from the bone stores (Hunter & Aub 1926-27). Certain physiological disturbances are also known to precipitate the release of stored lead from bone (Cantarow & Trumper 1944, King & Thompson 1961).

During an illness, bone lead is said to have produced clincial signs of lead poisoning, when the bone lead was released rapidly along with calcium (Brown & Tompsett 1945, Waldron & Stöfen 1974). When female mallard (Anas platyrhynchos) were exposed to lead, they had higher bone lead concentrations than males on the same dose regime (Finlay & Dieter 1978). The same was true of pigeons (Hutton 1980). Jordan & Bellrose (1951) reported that female ducks were also more susceptible to lead poisoning than males.

These observations pose the question whether egg formation in a female bird, with a high body burden of lead can subsequently lead to lead intoxication, as the lead is released from bone along with calcium reserves. This possibility has been mooted previously by other workers, but to my knowledge has not been investigated.

MATERIALS AND METHODS

Eight pigeons (Columba livia), four males and four females, were housed individually in experimental cages (46 x 53 x 38 cm high), with food, grit and water freely available. The food consisted of wheat, maize and maple peas in equal proportions by volume. The birds were allowed to acclimatise for seven days and during this time blood samples were taken to establish pre-experimental blood lead values.

Six birds, 3 males and 3 females, were then dosed daily for 10 days each time with 5 mg of lead nitrate incorporated in a gelatin capsule, giving a total dose of 50 mg.

Twenty-four hours after receiving the 10th dose the birds were paired by removing the central partition in the experimental cages. Dosing continued for a further 10 days, bringing the total dose of lead nitrate to 100 mg. A nest bowl was placed in each cage together with cut straw for nesting material. The remaining pair of birds acted as controls and as such received the same treatment but no lead nitrate. Blood samples were removed from a wing vein as far as possible every 24 hours after the final dose of lead nitrate. This was used to determine lead and calcium content.

The study was ended after 90 days; the surviving adult birds were killed by intravenous pentobarbitone injection. They were immediately examined and samples of liver, kidney and bone were removed for chemical analysis.

RESULTS

Between 5 and 7 days after dosing, all the birds began producing brilliant green faeces and showed signs of stress and inappetance, and

were increasingly easy to handle. One female (number six) died on day seven. Then the condition of the other birds began to improve, food consumption increased and the faeces began to lose the green colour and return to normal consistency. On day 14 bird number five, a male, the partner to bird number 6, also died. The initial blood lead levels (taken after the final dose of lead nitrate) were 2880, 2860, 2360, 1520, 3080 and 1360 µg/100 ml for birds 1 to 6 respectively (Figs. 1-6).

Thereafter blood lead values declined rapidly, and by day 10 values were between 240 and 940 µg/100 ml for the five remaining birds. These figures are between 6 and 25 times higher than the maximum acceptable level (MAL) for lead of 40 µg/100 ml blood established for swans by Birkhead (1982).

Bird number 6, the female which died, had an initial blood lead value of 1360 µg/100 ml and by day 7, when it died, the level had fallen to 600 µg/100 ml. Bird number 5, the male which died, had an initial blood lead level of 3080 µg/100 ml and by day 14, when it died, this level had declined to 424 µg/100 ml. The blood lead level of 600 µg/100 ml attained by bird number 6 was exceeded by four of the other five birds on day 7, and the blood lead level of bird number 5, 424 µg/100 ml, was exceeded by 3 of the remaining four birds on day 14.

A clutch of eggs were produced by birds 1 and 2 on day 10 and 12, a second clutch on days 30 and 33 and a third on days 58 and 60. Birds 3 and 4 also laid three separate clutches on 11 and 13, 22 and 24 and 64 and 66 days respectively. The control pair failed to lay during the study. All clutches were removed 3 days after production of the second egg.

Blood lead values remained fairly constant after the initial and rapid fall. On days 7 and 8, 28 and 29, 56 and 57, when serum calcium levels (Fig. 1) in bird number 1 reached a peak of between 19.8 and 21.0 mg per 100 ml, blood lead values were 1000 and 980, 750 and 762, 510 and 484 respectively. On days 6 and 8 and again on days 17 and 19, 60 and 62 when serum calcium levels (Fig. 4) in bird number four also peaked at between 18.4 and 22.0 mg per 100 ml, blood lead values were 250 and 262, 300 and 310, 240 and 236 respectively.

Liver and kidney levels of the four birds killed at the end of the study were below those normally accepted as indicative of lead poisoning in birds. Bone levels in both females exceeded those in males by a factor of approximately 1.5, and were indicative of chronic lead poisoning (Table 1).

Birds number 5 and 6 which died during the experiment, had lead levels 68.1 and 79.7 in liver and of 190 and 210 mg kg^{-1} in kidney which

exceeded the accepted lethal levels for lead poisoning in birds. Bone lead levels were 86.4 and 190 mg kg^{-1} respectively for birds number 5 and 6, again indicating acute lead poisoning.

Shells from the 12 eggs produced during the study had total lead levels of mean 2.81 g (range 1.31-2.88 g). Similar levels of lead have been found in the shells of American Kestrels dosed with metallic lead in their diet (Paltee 1984).

DISCUSSION

Throughout the study, blood lead levels remained above Birkhead's MAL for the whole 90 days. A lower dosing regime may have produced a plateau at a lower level. It has been suggested that lead will only disappear from the blood when the red cell dies, liberating the lead (Hursch & Soumela 1968). The life of an avian red cell is approximately 20 days and the observations, that blood lead levels in this experiment initially halved between 3 and 5 days, indicates that natural red cell death alone cannot be responsible for this fall. The rapid fall must have been due to the absorption of lead by the soft tissues and bone, before an equilibrium state was reached. There is also a transfer of lead from the plasma to the extra-vascular space.

Also a dynamic equilibrium exists between red cell and plasma lead, and extravascular and intra cellular lead (Stover 1959). The capacity of blood to carry lead in an equilibrium state must be dependent on red cell volume and lead dosing has been shown to reduce the red cell count to less than half its normal value (Coburn 1951).

Bone lead analysis on the two birds which died showed that deposition of lead in bone was rapid. Bone levels in the two females which survived to the end of the study had doubled when compared with the female which died, indicating a continued lead deposition. Finlay et al. (1978) noted that lead concentrations in wing bones of treated mallard were greater in females than in males and furthermore concentrations of lead in the wing bones of laying birds were more than 4 times those in non-layers. The bone lead values in the two remaining male pigeons had also increased but the values were lower than those in the females. No detectable reproductive effects were associated with lead dosing in this study. The differences between the bone lead levels in males and females demonstrates that a differential deposition of lead was occurring.

The elevated blood lead levels seen in female swans at certain times of year (Birkhead 1983) has previously been explained as a possible result of changing calcium metabolism. Increased demand for calcium

during eggshell formation can be satisfied from skeletal bone (Taylor & Moore 1954) or an increased uptake from the alimentary canal (Baltrop & Khoo 1976). Lead is preferentially stored in the bone and is said to follow similar metabolic pathways to calcium (Clarke & Clarke 1975). It is thought that (1) utilisation of bone calcium would also release lead or (2) increased intestinal absorbtion of calcium would increase absorption of lead. Quail began laying thin-shelled eggs 24 hours after receiving a reduced calcium diet (Osborn & Ogronoski, unpublished), implying that a majority of the calcium used in egg shell formation, in this species, is derived directly from the diet. Also laying domestic fowl, maintained on a low calcium diet, have been shown to deplete their cortical bone calcium rather than the medullary bone reserves (Taylor & Moore 1954).

It is argued that mobilisation of calcium from bone, either during disease (Brown & Tompsett 1945, Waldron & Stöfen 1974) or during normal demand for eggshell formation, would mobilise any absorbed lead (Birkhead 1983), but to date there is little evidence for either of these views. The levels of lead found in the eggshells were less than 1/100th of those found in the bone of the respective females. These results indicate that either (a) calcium and lead metabolism function independently of one another, (b) bone lead is unavailable during periods of high calcium demand especially eggshell formation, or (c)

eggshell calcium is derived directly from the diet and not from reserves in bone. Blood lead values remained constant during eggshell formation. Experiments carried out by Aub et al. (1926) showed that bone in vitro takes up lead in proportion to the calcium lost, but calcium would not release lead when the experiment was reversed. This fact must be taken into account when interpreting eggshell data. The two birds which died also show that, just prior to death, a time of maximum physiological upset, blood lead values remained constant. The often quoted work in support of this theory by Brown & Tompsett (1945) reports the effect of leukaemic hyperplasia on blood lead values from one human patient who had been, prior to the disease, exposed to elevated levels of lead. As the disease advanced, the patient began to suffer from lead poisoning, indicated by high blood lead values, and it was thought that the lead was derived from bone. At post-mortem, tissue levels of lead in the liver, kidney and brain of this patient were, in some cases, less than one tenth the values expected in the normal population (Waldron & Stöfen 1974) and did not indicate lead poisoning. However, femur levels were high. An alternative view, based on my findings coupled with the patient's extreme weight loss, is that the soft tissues in some way were depleted of lead, thus greatly elevating the blood levels.

Food contaminated with lead from roadside dust was thought to be responsible for the elevated lead levels in pigeons (Hutton 1980). And deposition of lead from motor vehicle exhausts also caused elevated levels of tissue lead in swallows (Grue et al. 1984) and starlings (Grue et al. 1986). Normally only 5% of ingested lead is retained (Evans & Moon 1981), but a high dietary demand for calcium may also increase the absorption and retention of lead.

It is possible that food contaminated with lead would place birds at risk during egg production. This experiment has shown that neither high bone nor high tissue levels of lead influenced blood lead values at a time of maximum calcium stress or death. It is from the blood that eggshell calcium is derived. This study further supports the results of the blood lead monitoring programmes carried out by Birkhead (1983) and Sears (1988). In their studies the fluctuating availability of lead, in the form of lead fishing weights, caused seasonal changes in swan blood lead values. My study has shown that high body burdens of lead and also changes in calcium physiology, had no detectable effect on blood lead values.

REFERENCES

Baltrop, D. & Khoo, H.E. (1976). The influence of dietary minerals and fat absorption of lead. Sci. Total Envir. **6**: 265-273.

Birkhead, M. (1982). Lead poisoning in the Mute Swan. Metals in Animals. ITE Symposium No.12 Editor D. Osborn. ITE Huntingdon.

Brown, A. & Tompsett, S.L. (1945). Poisoning due to mobilisation of lead from the skeleton by leukaemic hyperplasia of bone marrow. B.M.J. **2**, 764-765.

Cantarow, A. & Trumper, M. (1944). Lead poisoning. Williams & Wilkins, Baltimore.

Clarke, E.G. & Clarke, M.L. (1975). Veterinary Toxicology. 3rd Edition, London: Baillere.

Clarkson, T.W. & Kench, J.E. (1958). Uptake of lead by human erythro aytes in vitro. Biochemical Journal, **69**, 432-439.

Coburn, D.R., Metzler, D.W. & Treichler, R. (1951). A study of absorption and retention of lead in wild waterfowl in relation to clincial evidence of lead poisoning. J. Wildl. Mgmt., **15**: 186-192.

Evans, P.R. & Moon, S.J. (1981). Heavy metals in northern England: Environmental and Biological Aspects. Ed. P.J. Say and B.A. Whitton, Univ. of Durham. ISBN 0-903569-01-9.

Finlay, M.T., Dieter, M.P. & Locke, L.N. (1976). Lead in tissues of mallard ducks dosed with two types of lead shot. Bull. Environ. Contam. Toxicol. **16**, 261-269.

Finlay, M.T. & Dieter, M.P. (1978). Influence of laying on lead accumulation in bone of mallard ducks. J. Toxicol. Environ. Health **4**: 123-129.

Grue, C.E., O'Shea, T.J. & Hoffman, D.J. (1984). Lead concentrations and reproduction in highway-nesting barn swallows. Condor, **86**: 383-389.

Grue, C.E., Hoffman, D.J., Beyer, W.N. & Fraison, W.N. (1986). Lead concentrations and reproductive success in european starlings. Sturnus vulgaris nesting within highway roadside verges. Environ. Pollut. **42**: 157-182.

Harrison, R.M. & Laxen, D.P.H. (1981). Lead pollution causes and control. Chapman Hall. ISBN 0-412-16360-8.

Hunter, D. & Aub, J.C. (1926-27). Quarterly Journal of Medicine **20**, 123.

Hursch, J.B. & Soumela, J. (1968). Absorption of 212 Pb from the Gastrointestinal Tract of man. Acta Radiologica Ther. Physica Biologica **7**, 108-120.

Hutton, M. (1980). Metal contamination of feral pigeons Columba livia from the London area: Part 2 - biological effects of lead exposure. Envrion. Pollut., **22**, 281-293.

Jordan, J.S. & Bellrose, F.C. (1951). Lead poisoning in wild waterfowl. Ill. St. nat. Hist. Surv. Biol. Notes No.26.

King, E. & Thompson, A.R. (1961). The measurement of lead absorption in industry. Annals of Occupational Hygiene **3**, 247-263.

Pattee, O.H. (1984). Eggshell thickness and reproduction in American Kestrels exposed to chronic dietary lead. Arch. Environ. Contam. Toxicol., **13**: 29-34.

Rowland, R.E. (1966). Exchangable Bone Calcium. Clin. Orthop., **49**, 233-248.

Stover, B.J. (1959). Proceedings of the Society for Experimental Biology and Medicine **100**: 269.

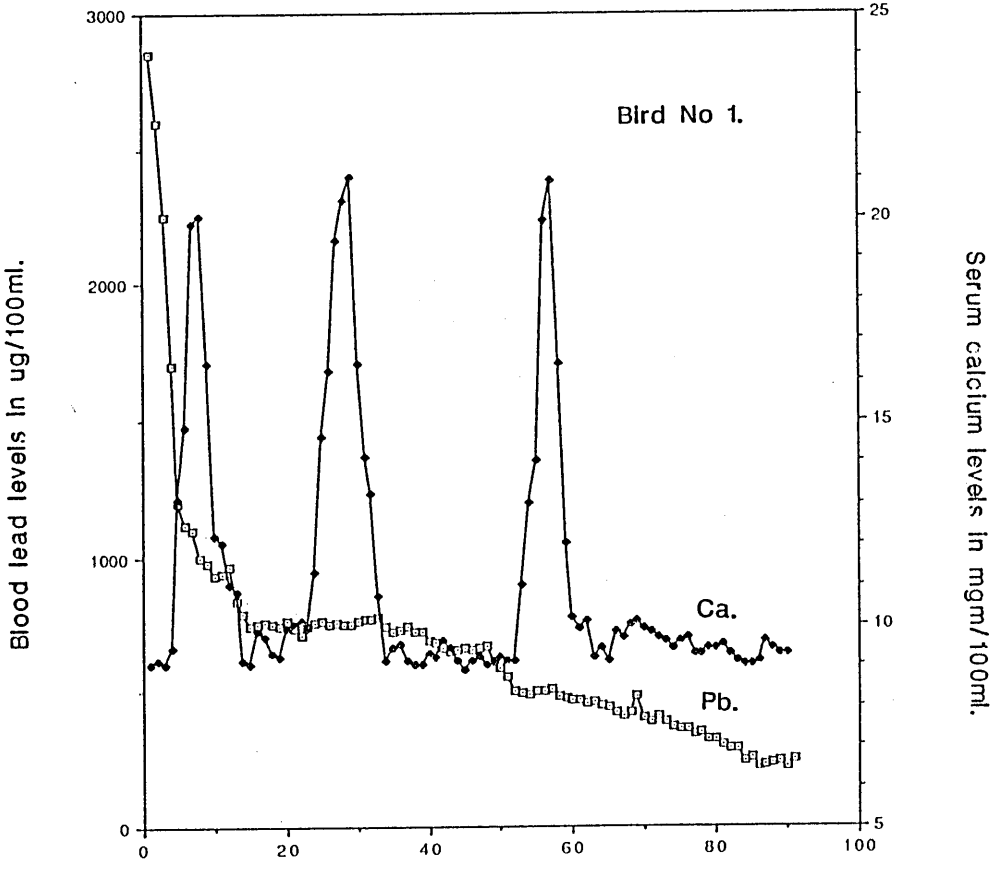
Taylor, T.G. & Moore, J.H. (1954). Skeletal depletion in hens laying on a low-calcium diet. Brit J. Nutr., 8, 112-124.

Waldron, H.A. & Stöfen, D. (1974). Sub-clinical lead poisoning. Academic Press. ISBN 0-12-671650-1.

TABLE 1

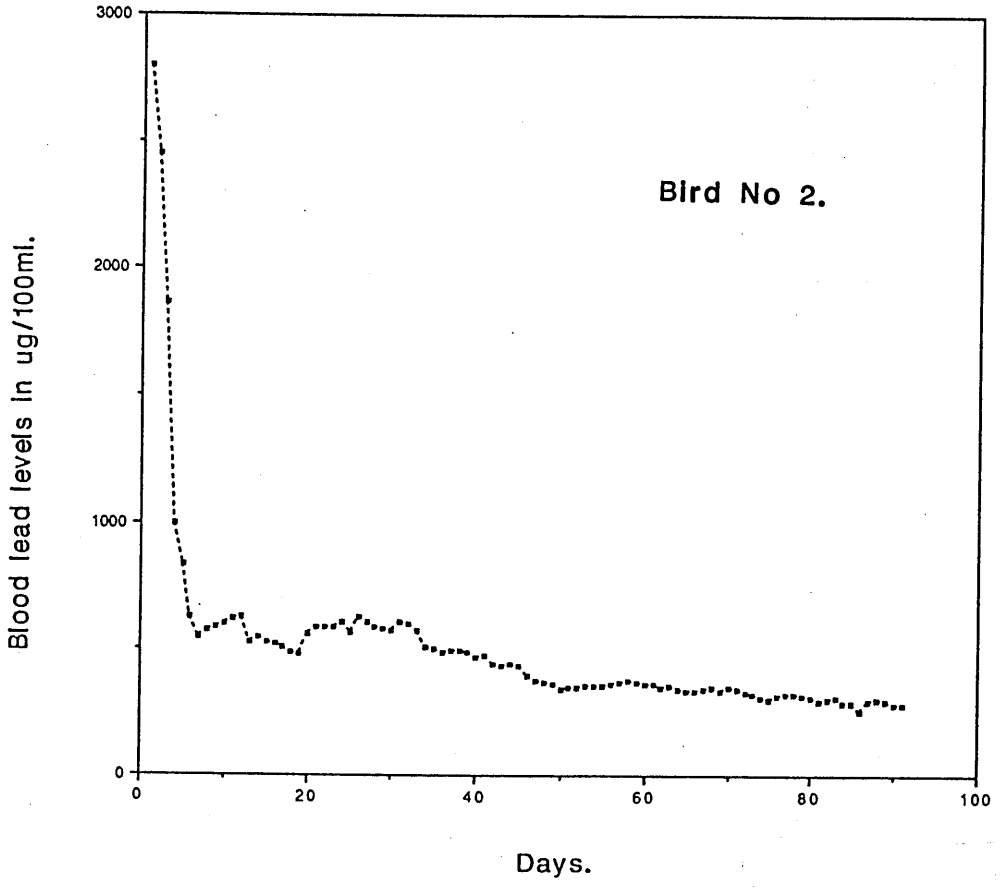
Lead levels (mg kg-1 dry weight) in liver, kidney and bone, of pigeons at post-mortem.

Bird Number (sex in brackets)		1 (f)	2 (m)	3 (m)	4 (f)	5 (m)	6 (f)
Tissue	Liver	20.6	14.2	21.4	17.2	68.1	79.7
	Kidney	78.1	68.2	90.0	63.1	190	210
	Bone	320	194	210	348	86.4	290



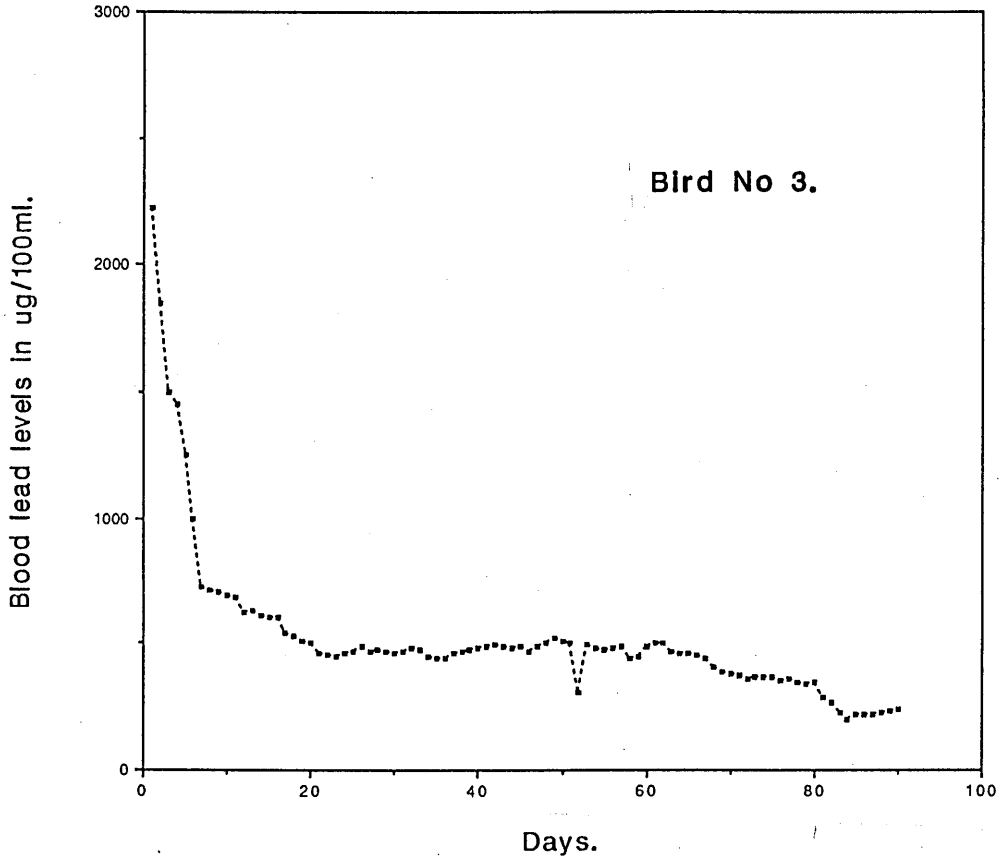
Blood lead and serum calcium levels in pigeons on successive days after dosing.

Fig 1.



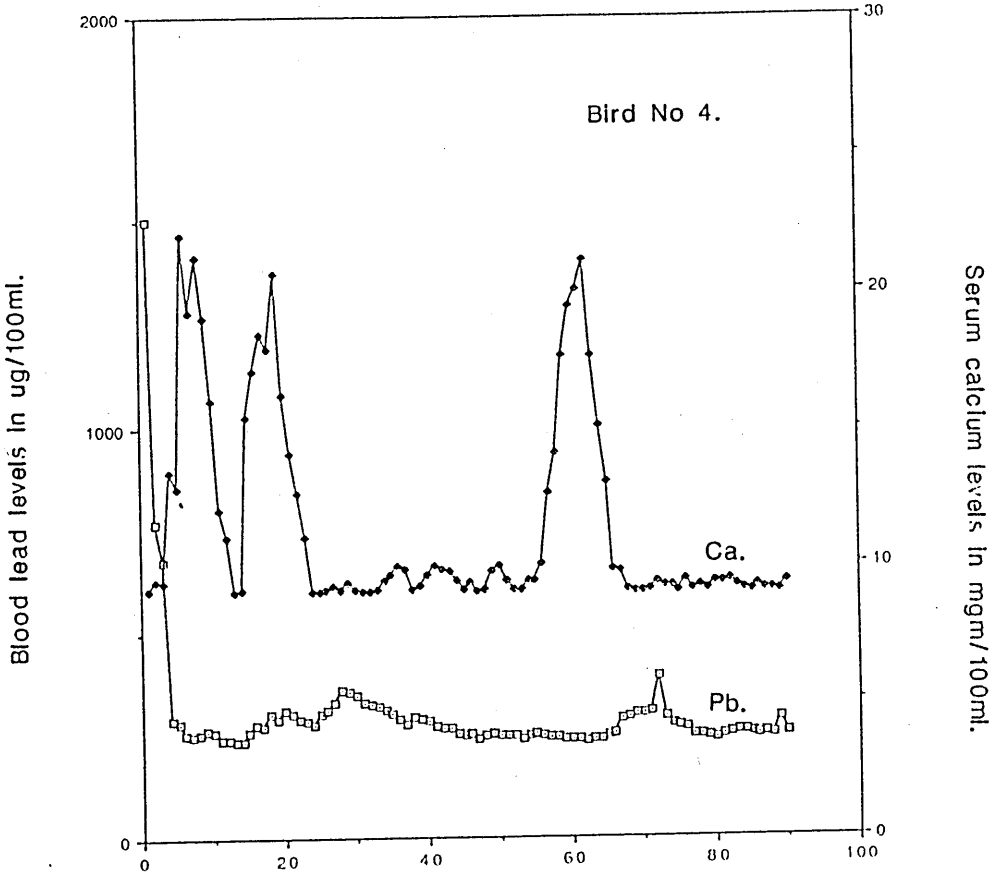
Blood lead levels in pigeons on successive days after dosing.

Figure 2



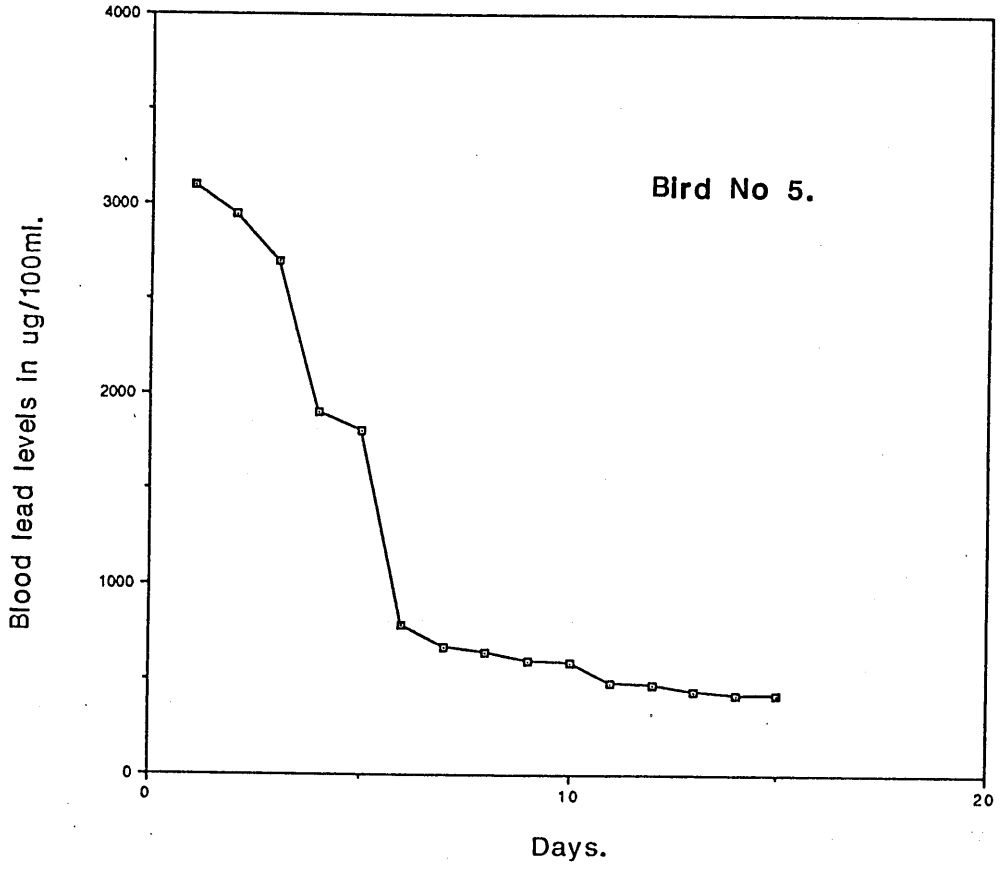
Blood lead levels in pigeons on successive days after dosing.

Figure 3



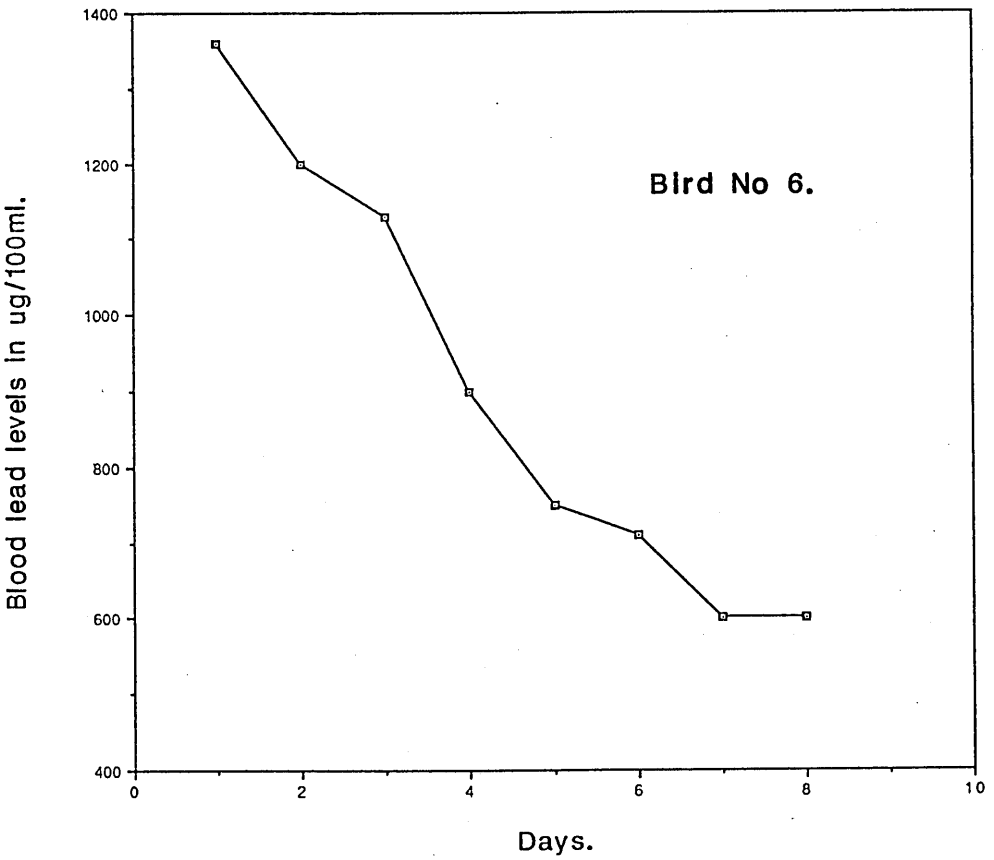
Blood lead and serum calcium levels in pigeons on successive days after dosing.

Fig 4.



Blood lead levels in pigeons on successive days after dosing.

Figure 5



Blood lead levels in pigeons on successive days after dosing.

Figure 6

Chapter 5

Biliary and Intestinal Excretion of Lead in Lead Poisoned Mute Swans (*Cygnus olor*)

INTRODUCTION

The principal routes of elimination of lead from the body are via the faeces and urine. Urinary excretion accounts for a small portion of absorbed lead (Blaxter 1950). Lead can also be excreted in the milk of lactating animals (Kehoe et al. 1940). Most of the lead present in the faeces represents ingested lead which has passed unabsorbed through the intestinal tract. However, some of this could originate from other sources, namely the bile and pancreatic juices, succus entericus or the intestinal wall. Observations so far favour biliary excretion. In an experiment with a single sheep, 7.5% of a lead acetate injection was eliminated in 6 days, 81% of which was excreted in the bile, while faecal and urine excretion made up the other 19% (Blaxter & Cowie 1946). Bile in this experiment was surgically drained for analysis to prevent mixing with the gut contents.

Swans and other waterfowl suffering from lead poisoning invariably have grossly distended gall bladders and green stained vent areas (Simpson et al. 1979; Jordan & Bellrose 1951; Chapter 2). This project was

initiated to determine whether increased biliary activity played an important role in the excretion of lead in lead-poisoned swans (Cygnus olor).

MATERIALS AND METHODS

Seven swans were chosen whose immediate cause of death was not lead poisoning but whose gizzards contained anglers' lead weights and whose gut function seemed normal (no impaction of the oesophagous, proventriculus or gizzard and food present in the alimentary canal and also the presence of normal-looking faeces in the cloaca). A post-mortem was carried out, and liver, kidney, bile, bone, faeces and gut contents were removed for chemical analysis. The gastrointestinal tract was removed intact from the junction of the gizzard and duodenum to 5 cm past the junction of the rectum with the caeca. Fifteen centimetre sections of gut starting at the duodenum and ending at the ileum were removed at approximately 50 cm intervals along the gut length to give five specimens. The contents from these sections, together with faecal samples and the pooled contents of both caecae were removed for individual chemical analysis. Complete faecal samples were present in one bird and, together with the remaining gut contents, were removed for chemical analysis and calculation of total gut load of lead.

Five birds shown to carry only background levels of lead which died in road traffic accidents and two which died after colliding with overhead power cables were used as controls.

The gall bladder was removed intact from all the birds and bile volume and pH were determined.

RESULTS

Gall bladder volumes in control birds were significantly lower (mean 21.2 ml, range 18.6-25.8 ml) than in lead poisoned birds (mean 29.7 ml, range 27.0-38.2 ml) ($t = 6.38$ DF12 $P<0.0001$). Bile pH was not significantly different between control birds (mean pH 5.8, range 5.6-6.5) and lead-poisoned birds (mean pH 6.0, range 5.8-6.4) ($t = 0.80$ DF12 P 0.4392). Results of analysis of liver, kidney, bile and bone for lead are shown in Table 1.

The relatively low bone levels of lead, when compared with the higher levels in the liver and kidney, indicate that all the lead poisoned swans were suffering from acute lead poisoning (Allcroft 1951).

All seven lead poisoned birds had detectable levels of lead in the

bile: mean 13.6 mg kg^{-1}, range 8.4-22.4 mg kg^{-1}. Mean levels of lead in the duodenal contents were 398 mg kg^{-1} (range 239-620 mg kg^{-1}). Levels were lower in the three consecutive samples taken from the midgut area, with mean values of 370 mg kg^{-1} (range 234-618 mg kg^{-1}), 122 mg kg^{-1} (range 55-235 mg kg^{-1}) and 146 mg kg^{-1} (range 51-297 mg kg^{-1}) respectively. Levels then increased in the ileum (mean 210 mg kg^{-1}, range 120-400 mg kg^{-1}) and caecal contents (mean 540 mg kg^{-1}, range 205-1010 mg kg^{-1}) to exceed the values in the midgut contents. In every bird faecal lead levels were lower than their respective duodenal contents by between 2 and 18% (mean 359 mg kg^{-1}, range 208-522 mg kg^{-1}) but were higher than values seen in the midgut samples (Fig. 1). Analysis of the single complete gut and faecal samples revealed a total lead burden of 1436 g. Levels of lead in the bile and gut samples of all the control birds was <5 mg kg^{-1}.

DISCUSSION

Previous research has reported that much of the lead in the faeces of humans (Karhauser 1973), rats (Quarterman & Morrison 1975), sheep and rabbits (Blaxter 1950) had passed through the gut unabsorbed. Evans & Moon (1981) calculated that Curlew (Numenius arquata) retained only 5% of the lead from their diet of ragworms (Nereis diversicolor). This present study on swans with a high degree of lead contamination shows

that, whilst not disputing any of these figures, much of the faecal lead (64%) originates from lead absorbed and then secreted back into the gut lumen.

It has been suggested that lead can pass through the gut wall attached to proteins which are then proteolysed to liberate lead (Massione 1941, Witchii 1964). The gut wall has also been shown to contain small quantities of lead (Cikrt 1972), and biliary excretion is the main route of lead elimination from the body (Castellino et al. 1966, Cikrt 1972). Longcore et al. (1974) found significant quantities of lead in the gall bladders of 36 dead or sacrificed ducks. However, Castellino et al. (1966) could not be certain that lead appeared in the gastro-intestinal tract solely from the bile. Studies carried out by Cikrt (1972) indicate that lead excreted by the gastro-intestinal tract, mainly from the upper segments, has little effect on overall faecal levels of lead. Lead contained in the bile would elevate the lead level in the gut contents in the duodenal area of the alimentary tract. This study has shown that following movement of these contents through the gut, levels of lead decline and then become elevated again in the lower gut. Biliary excretion of lead would seem in this case not to be a major contributor to faecal lead levels. It has been proposed that lead and calcium follow a similar metabolic pathway (Clarke & Clarke 1975). In rats, following duodenal absorption of

calcium, there is a secretion of calcium back into the lumen of the ileum (Sernka & Borle 1969). Secretions of lead may follow this same pathway.

Caecal function in birds has been extensively studied (Moss & Parkinson 1972, Moss & Parkinson 1975, Gassaway 1976, Hanssen 1979). Dietary fibre digestion is thought to be a major caecal function, but absorption of water both from gut contents and urine is also thought to take place (Duke 1977, Sturkie 1976). This could account for the high levels of lead seen in the caecal contents, as it would be concentrated by fibre digestion. Avian urine is known to be forcibly mixed with gut contents in the cloaca, ileum and caeca (Akester et al. 1967, Skadhauge 1968). Therefore, the proportion of avian faecal lead which is urinary in origin is not known and cannot be calculated from the results of this experiment.

Conrad & Barton (1978) laparotomised 12 rats, half had their bile ducts ligated, the other group did not. Intestinal segments were isolated with umbilical tape and the incision closed. Four hours after an injection of radio-labelled lead in saline, all the animals were killed and the individual gut segments were removed intact. Chemical analysis revealed (1) between 10.7 and 13.2% of body lead was in the gut segments, (2) there was more lead in the duodenum of the rats with

intact bile ducts than in those with ligated ducts, and (3) there was more lead in the ileum than in the jejunum in both groups.

These results are consistent with mine in that biliary excretion does occur in swans, but the lower gut contributes significant amounts of lead back into the gut lumen for subsequent egestion. In other words, a high proportion (64%) of faecal lead derived from lead metal has not passed through the alimentary tract unabsorbed. These findings may also be of value in the treatment of lead poisoned swans in that the presence of faecal lead is a reliable indicator of the bodys ability to eliminate lead back into the lumen of the gut for subsequent excretion.

REFERENCES

Akester, A.R., Anderson, R.S., Hill, K.J. & Osbaldiston, G.W. (1967). A radiographic study of urine flow in the domestic fowl. Br. Poult. Sci. **8**, 209-212.

Allcroft, R. (1951). Lead poisoning in cattle and sheep. Vet. Rec. Vol 63, 583-590.

Blaxter, K.L. & Cowie, A.T. (1946). Excretion of lead in the bile. Nature **157**, 588.

Blaxter, K.L. (1950). Lead as a nutritional hazard to farm livestock. II The absorption and excretion of lead by sheep and rabbits. J. comp. Pathol. **60**, 140-159.

Castellino, N., Lamanna, P. & Grieco, B. (1966). Biliary excretion of lead in the rat. Brit. J. industr. Med. **23**, 237-239.

Cikrt, M. (1972). Biliary excretion of 203 Hg, 64 Cu, 52 Mn and 210 Pb in the rat. Brit. J. industr. Med. **29**, 74-80.

Clarke, E.G. & Clarke, M.L. (1975). Veterinary Toxicology 3rd ed. London: Baillere.

Conrad, M.E. & Barton, J.C. (1978). Factors affecting the absorption and excretion of lead in the rat. Gastroenterology **74**, 731-740.

Duke, G.E. (1977). Avian digestion. In Dukes Physiology of Domestic Animals. Ed. Swenson, M.J., Cornell University Press. Ittaca NY.

Evans, P.R. & Moon, S.J. (1981). Heavy metals in Northern England: Environmental and Biological Aspects. Ed. D.J. Say and B.A. Whitton, University of Durham. ISBN 0-903569-01-9.

Gasaway, W.C. (1976). Cellulose digestion and metabolism by captive rock ptarmigan. Comp. Biochem. Physiol. **54**, 179-182.

Hanssen, I. (1979). A comparison of the microbiological conditions in the small intestine and ceca of wild and captive willow grouse (Lagopus lagopus lagopus). Acta. Vet. Scand. **20**, 365-371.

Jordan, J.S. & Bellrose, F.C. (1951). Lead poisoning in wild waterfowl. Natural History Survey Division, State of Illinois. Biological Notes No.26.

Karhauser, L.R. (1973). Intestinal absorption of lead. Nucl. Sci. Abs. **27**, 21066-81.

Kehoe, R.A. (1961). The metabolism of lead in man in health and disease. Present hygienic problems relating to the absorption of lead. Jl. R. Inst. publ. Hlth. Hyg. **24**, 177-203.

Longcore, J.R., Locke, L.N., Bagley, G.E. & Andrews, R. (1974). Significance of lead residues in Mallard tissues. Special Scientific Report Wildlife No.182. Washington DC.

Massione, R. (1941). Il piombo nella bile di individii normali e saturnini. Medicina del Lavoro **32**, 149.

Moss, R. & Parkinson, J.A. (1972). The digestion of heather (Calluna vulgaris) by Red grouse (Lagopus lagopus scoticus). Br. J. Nutr. **27**, 285-298.

Moss, R. & Parkinson, J.A. (1975). The digestion of bulbils (Polygonum viviparum L.) and berries (Vaccinium myrtillus L. and Eripetrum sp.) by captive ptarmigan (Lagopus mutus). Brit. J. Nutr. **33**, 197-206.

Quartermann, J. & Morrison, J.N. (1975). The effects of dietary calcium and phosphate on the retention and excretion of lead in rats. Brit. J. Nutr. **34**, 351-362.

Sernka, T.J. & Borle, A.B. (1969). Calcium in the intestinal contents of rats on different calcium diets. Proc. Soc. Exp. Bid. N.Y. **131**: 1420-1423.

Simpson, V.R., Hunt, A. & French, M.C. (1979). Chronic lead poisoning in a herd of mute swans. Environ. Pollut. **18**, 187-202.

Skadhauge, E. (1968). Cloacal storage of urine in the rooster. Comp. Biochem. Physiol. **24**, 7-18.

Sturkie, P.D. (1976). Avian Physiology, 3rd Edition. Springer-Verlag, N.Y.

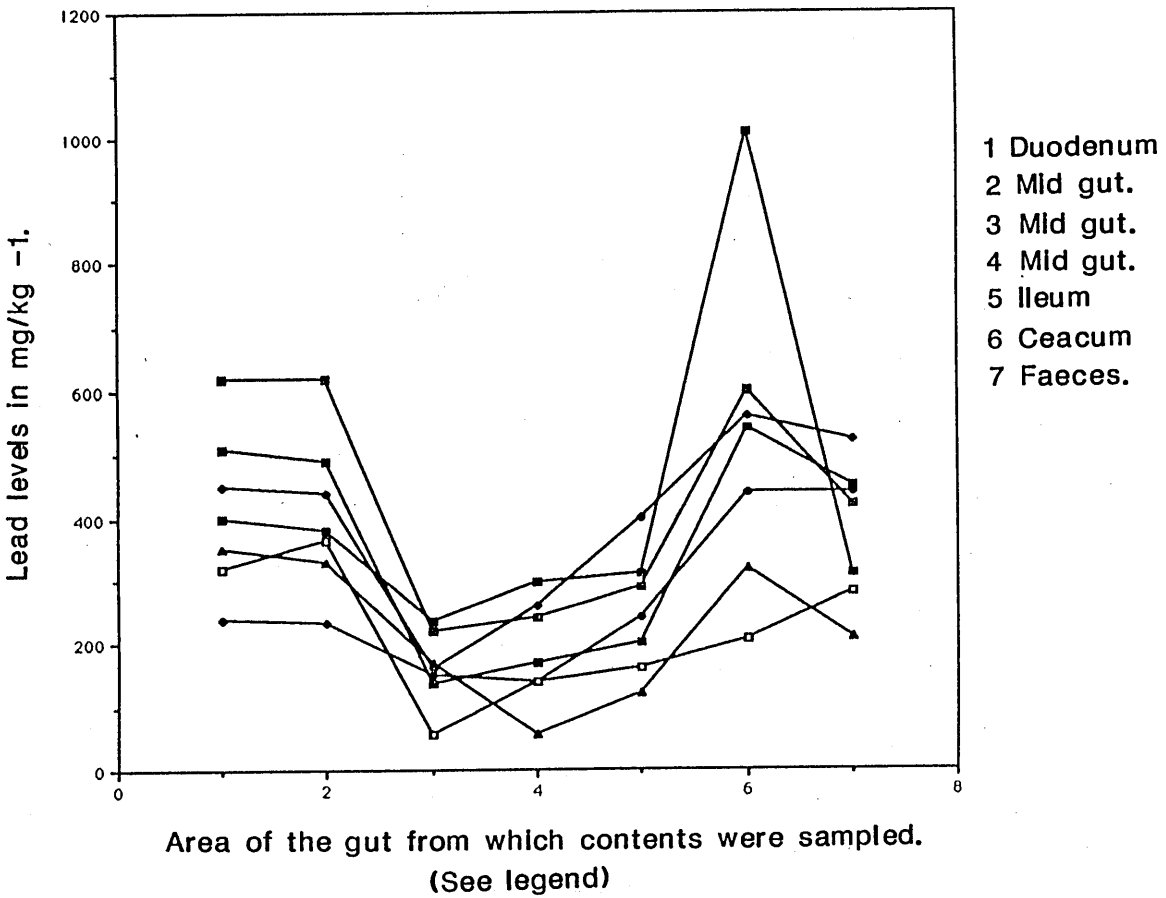
Waldron, H.A. & Stofen, D. (1974). Sub-clinical lead poisoning. Academic Press, ISBN 0-12-671650-1.

Witschi, H.P. (1964). Tier experimentelle unter suchungen zur enteralen Bliansscheidung. Archiv fur Gewerbepathologie und Gewerbehygiene **20**, 449.

TABLE 1

Results of liver, kidney, bone and bile analysis for lead in mute swans expressed on a Dry Weight basis mg/kg-1

		Liver	Kidney	Bone	Bile
	1	42.6	72.9	130	14.6
	2	39.8	163.4	94.0	8.4
Lead	3	24.4	30.0	136	22.4
Contaminated	4	20.6	147.9	111	9.1
Birds	5	32.6	82.4	96.7	3.6
	6	40.9	250	85.4	2.1
	7	51.3	139	126	4.8
	8	10.6	28.6	40.1	N/D
	9	8.2	19.4	31.4	N/D
Control	10	9.4	16.2	20.0	N/D
	11	3.7	21.0	29.4	N/D
	12	14.6	13.7	18.4	N/D
	13	22.0	29.6	31.0	N/D
	14	9.1	26.0	20.0	N/D



Analysis of contents taken from various parts of the gut of Mute Swans for lead.

Figure 1.

Chapter 6

Influence of Thiamin Administration on Mallard Ducks (*Anas platyrhynchos*) following ingestion of Lead Shot

INTRODUCTION

Chelation therapy is widely used in the management of metallic poisoning and is the treatment of choice for lead intoxication. Three drugs are in common use, namely EDTA administered as its Ca - chelate, British Anti Lewisite (BAL) and D - penicillamine (Waldron & Stöfen 1974).

Bratton (1981) discusses the therapeutic potential of thiamin (Vitamin B_1) in the treatment of lead poisoning. It is, he says, "a naturally occuring compound, which readily enters the cell, is not toxic and effectively interacts with Pb to reduce the latter's toxic potential".

The treatment of lead poisoned birds after they have ingested lead gunshot or fishing weights by bird hospitals and veterinary surgeons is now common practice and a drug with no serious or detectable side effects has considerable attraction.

The prolonged use of any chelation drugs may also deplete or render inactive essential metals (Hammond, 1971). Ca-EDTA administered orally

will also increase the intestinal absorption of lead (Reiders 1960, Kehoe 1955, Chisholm 1968). Lead absorption from the bowel may be promoted after intravenous administration of Ca-EDTA (Byers 1959), and the same is true after parenteral administration of both Ca-EDTA and BAL, Jugo et al. (1975). Chelating agents are excreted in the bile and thus insoluble lead present in the gut would be rendered soluble after chelation therapy. Toxic symptoms in children with lead paint chips present in the gut are exacerbated following chelation therapy (Waldron & Stöfen 1974). Chisholm (1968) noted that, in some cases of lead poisoning, there was an increased deterioration in patients after chelation therapy had begun.

It is evident that before lead poisoned birds are treated with these chelating agents lead fragments in the form of gunshot or fishing weights present in any part of the alimentary tract must ideally be removed first.

There is some dispute over the source of excreted lead following Ca-EDTA therapy. Miller (1959) states that the soft tissue lead is depleted rather than the bone lead, and Castellino and Aloj (1965) support this theory. However, Hammond (1971) maintains that the source of excreted lead is the bone stores, and was able to confirm this in both rats and rabbits.

A deer, five cows, one goat and four dogs which had ingested lead improved or totally recovered when treated with injections of thiamin (Bratton 1981). The author carried out experiments using twenty five calves to determine the effect of thiamin on lead poisoning under experimental conditions. In his first experiment, he used fifteen male calves divided into three groups of five animals, giving control, lead-dosed and lead-dosed plus thiamin groups. The control and the lead-dosed plus thiamin groups remained clinically normal, whilst in the group dosed with lead only, deaths and abnormal behaviour occurred. The second experiment with ten calves confirmed these findings. Encouraged by these results, I did the following experiment to determine the effect of thiamin on lead dosed mallard (Anas platyrhynchos).

METHODS

Twelve adult mallards, eighteen months old and weighing 1079-1286 gr were obtained from a registered game dealer. The ducks were maintained after wing clipping in a grass enclosure measuring 10 m x 20 m, with food, chick crumbs and wheat, water and grit freely available. The ducks were allowed to acclimatise for eight days and they were then X-rayed and found to be free of ingested foreign objects.

The birds were then divided into three groups. Group I control, Group II lead-dosed and Group III lead-dosed plus daily injections of thiamin. The weighed lead dose consisted of 5 No.6 lead shot incorporated in a gelatin capsule, which was force fed to the birds in Groups II and III. The ducks in Group III also received daily intra-muscular injections of thiamin dissolved in physiologically normal saline (Thiamin Hydrochloride (Vit B_1) Sigma Chemical Co.) at a strength of 2.5 mgm/Kg body weight. Thiamin treatment began one hour after dosing with lead shot and injections were carried out at the same time of day during the experiment.

All the birds in the control group gained weight during the experiment by an average of 128 g (range 120-139 g). Both dosed groups lost weight, group 2 by an average of 194 g (range 170-240 g) and group 3 by 210 g (range 180-268 g). Mean weight loss in the two dosed groups was not significantly different.

Twenty four hours after dosing the birds were X-rayed to determine how many of the original shot had been retained. It transpired that none of the shot had been voided.

The study was ended after 12 days; the surviving ducks were killed by cervical dislocation. They were immediately post-mortemed and samples

of liver, kidney and bone were removed for chemical analyses. Lead weights remaining in the gizzard were removed, cleaned and reweighed.

RESULTS

All the birds in Groups II and III deteriorated during the study. One bird from Group II died after 5 days, two more birds died after eight days one from Group II the other from Group III. Because of the condition of the remaining birds in both Groups II and III the experiment was terminated after 12 days. Signs of lead poisoning were evident in both lead dosed groups from the 4th day. The birds had difficulty in walking and occasionally fell over and green stained feathers could be seen around the vent.

Original and eroded shot weights together with daily erosion rate are shown in Table 2.

Analysis of various tissues for lead (Table 1) gave control (Group 1) means of 2, and means of 3.85 $\mu g\ g^{-1}$ (range 2.0-5.6 $\mu g\ g^{-1}$) and 31.2 $\mu g\ g^{-1}$ (range 28-35 $\mu g\ g^{-1}$) in liver, kidney and bone respectively. The lead dosed birds (Group 2) had means of 145 $\mu g\ g^{-1}$ (range 123-186 $\mu g\ g^{-1}$), 223 $\mu g\ g^{-1}$ (range 128-334 $\mu g\ g^{-1}$) and 90.7 $\mu g\ g^{-1}$ (range 63.6-137 $\mu g\ g^{-1}$) in the same three tissues. While

the thiamin-treated birds (Group 3) had levels of 168 µg g^{-1} (range 140-228 µg g^{-1}), 304 µg g^{-1} (range 96-560 µg g^{-1}) and 31.8 µg g^{-1} (range 9.4-46 µg g^{-1}).

The concentration of lead in the liver and kidney were not significantly different between the two dosed groups ($t = 1.074$, DF9, $P > 0.05$ and $t = 0.870$, DF9, $P > 0.05$). But concentrations in bone were significantly lower in the thiamin-treated birds than the lead dosed group receiving no thiamin treatment ($t = 3.952$, DF9, $P > 0.01$). The thiamin-treated birds were no different in this respect from the controls ($t = 0.055$, DF9, $P > 0.05$). The liver and kidney levels in both dosed groups were above the levels normally associated with lead poisoning in birds (Clarke & Clarke 1975). The mean blood lead for all 12 ducks during the period of settling in was 2.8 µg/100 ml blood (range 0.8 to 3.2 µg /100 ml). The maximum blood lead level attained in Group II was 394 µg /100 ml on day 9 and in Group III 394 µg/100 ml on day 12 (Figure 1).

Birds 7, 8, 9, 10 and 12 showed a steady blood lead increase after dosing until day 6, 6, 6, 8 and 8 respectively. Following this, the blood lead levels became erratic.

The blood lead levels of the three birds which died were 374, 360 and 380 µg/100 ml in the previous 24 hours. The remaining birds had blood

lead levels which exceeded these concentrations at some time during the experiment. The eight dosed birds retained all the original dose of lead pellets, erosion rates are shown in Table 1 and are calculated on the amount of lead eroded per day assuming a constant rate. The three birds which died (number 5 on day 5, number 6 and 11 on day 8) had eroded 93.4, 108 and 89.4 mg respectively of lead. Bird number 12 which had eroded 4 times as much lead as bird number 7 had similar blood lead concentrations to number 7 (394 and 364 µg/100 ml) by day 12. The blood lead levels of the two dosed groups rose within 24 hours above the maximum accepted levels (40 µg/100 ml blood) for swans in the wild (Birkhead 1983). In the control group, blood lead levels displayed a maximum of 3.2 µg/100 ml (range 0.8 to 3.2 µg/100 ml). A similar pattern of blood lead levels was noted by Allcroft (1951) when calves were dosed with the lead ore (galena) or lead metal alone.

Because of the interaction of other elements with lead (Six & Goyer 1970, Barltop & Khoo, 1975), whole blood measurements of calcium, magnesium and zinc were taken. Only minor variations were observed but were not statistically different, and are not shown here.

DISCUSSION

The initial rapid availability of lead, followed by a slowing down of

erosion, could account for the pattern of blood lead changes observed. It is known that erythrocyte count drops when birds are exposed to lead, to as much as a third of the normal value within 6 days (Coburn 1951). In this same study red cell counts became erratic in some birds a few days prior to death. These observations would further accentuate the effect of a decline in lead erosion rate and give rise to the pattern of blood lead levels seen in my experiment.

Immediately after dosing, shot erosion would presumably proceed rapidly due to gizzard activity and the high acidity of the gizzard juices (pH 2.4). As lead intoxication progresses, the gizzard eventually loses its grinding ability and the lead weights are then eroded only by the acid; also as the shot gets progressively smaller the erosion rate would further decline due to reduced surface area.

In this experiment thiamin treatment had no obvious beneficial effects on lead poisoning. There was no significant reduction in tissue levels of lead in the thiamin- and lead-treated ducks, over the lead-treated ducks and all the lead-dosed birds showed clinical signs of lead intoxication. This contrasts with the findings of Bratton (1981) who demonstrated a lowering of tissue lead levels in calves treated with thiamin and also observed no clinical or pathological signs of lead intoxication in the vitamin treated group.

In my study accumulation of lead in the bone was suppressed in the thiamin-treated group when compared with the lead-treated group receiving no thiamin. There was no significant difference in bone lead levels between the control and thiamin treated groups. This implies that thiamin in some way blocks bone deposition of lead or enhances transportation of lead from bone to soft tissue. These findings were also in opposition to those of Bratton (1981), who found that bone levels were higher in calves treated with thiamin than in control calves. He also quotes unpublished data showing that thiamin elevated bone levels in chicks. The single deer treated by Bratton (1981) which responded to thiamin dosing but relapsed some time after receiving its last injection, was found to have "a lead source" which was removed; after further treatment the deer recovered. In my study the lead source in the ducks remained in the gut, but I failed to observe any beneficial effect of thiamin administration. However, coupled with lead removal from the alimentary canal and the use of a chelating agent the ability of thiamin to prevent bone accumulation of lead may prove beneficial in further experiments.

REFERENCES

Allcroft, R. (1951). Lead poisoning in cattle and sheep. Vet Record, **37, 63,** 583-590.

Baltrop, D. & Khoo, H.E. (1976). The influence of dietary minerals and fat absorption of lead. Sci. Total Envir. **6**, 265-273.

Birkhead, M. (1983). Lead levels in the blood of mute swans Cygnus olor on the River Thames. J. Zool., Lond. **199**, 59-73.

Bratton, G.R., Zmudzki, J., Bell, M.C. & Warnock, L.G. (1981). Thiamin (Vitamin B_1) Effects on lead intoxication and deposition of lead in tissues: Therapeutic Potential. Tox. Appl. Pharm. **59**, 164-172.

Byers, R.K. (1959). Lead poisoning. Review of the literature and report on 45 cases. Pediatrics **23**, 585-603.

Castellino, N. & Aloj, S. (1965). Effects of calcium sodium ethylene-diaminoletra-acetate on the kinetics of distribution and excretion of lead in the rat. Brit. J. Ind. Med. **22**, 172-180.

Chisholm, J.J. (1968). The use of chelatin agents in the treatment of acute and chronic lead intoxication in childhood. J. Pediat. **73**, 1-38.

Clarke, E.G. & Clarke, M.L. (1975). Veterinary Toxicology 3rd ed. London: Baillere.

Coburn, D.R., Metzler, D.W. & Tp Treichler, R. (1951). A study of absorption and retention of lead in wild waterfowl in relation to clinical evidence of lead poisoning. J. Wild. Mgmt. **15**: 186-192.

Hammond, P.B. (1971). The effects of chelating agents on the tissue. Distribution and excretion of lead. Tox. and Appl. Pham. **18**, 296-310.

Jugo, S., Maljkovic, T. & Kostial, K. (1975). Influence of chelating agents on the gastrointestinal absorption of lead. Tox. Appl. Pharm. **34**, 259-263.

Kehoe, R.A. (1955). Misuse of edathamil calcium disodium for prophylaxis of lead poisoning. J. Amer. Med. Ass. **157**, 341-342.

Miller, L.H. (1959). EDTA therapy in persons with excessive lead absorption from industrial exposure. Ind. Med. Surg. **28**, 144-147.

Reiders, F. (1960). Current concepts in the therapy of lead poisoning. In. Metal-binding in Medicine (M.J. Seven Ed.), pp.143-145. Lippincott, Philadelphia.

Six, K.M. & Goyer, R.A. (1970). Experimental enhancement of lead toxicity by low dietary calcium. J. Lab. Clin. Med., **76**, 933-942.

Waldron, H.A. & Stofen, D. (1974). Sub-clinical lead poisoning. Academic Press, London.

TABLE 1

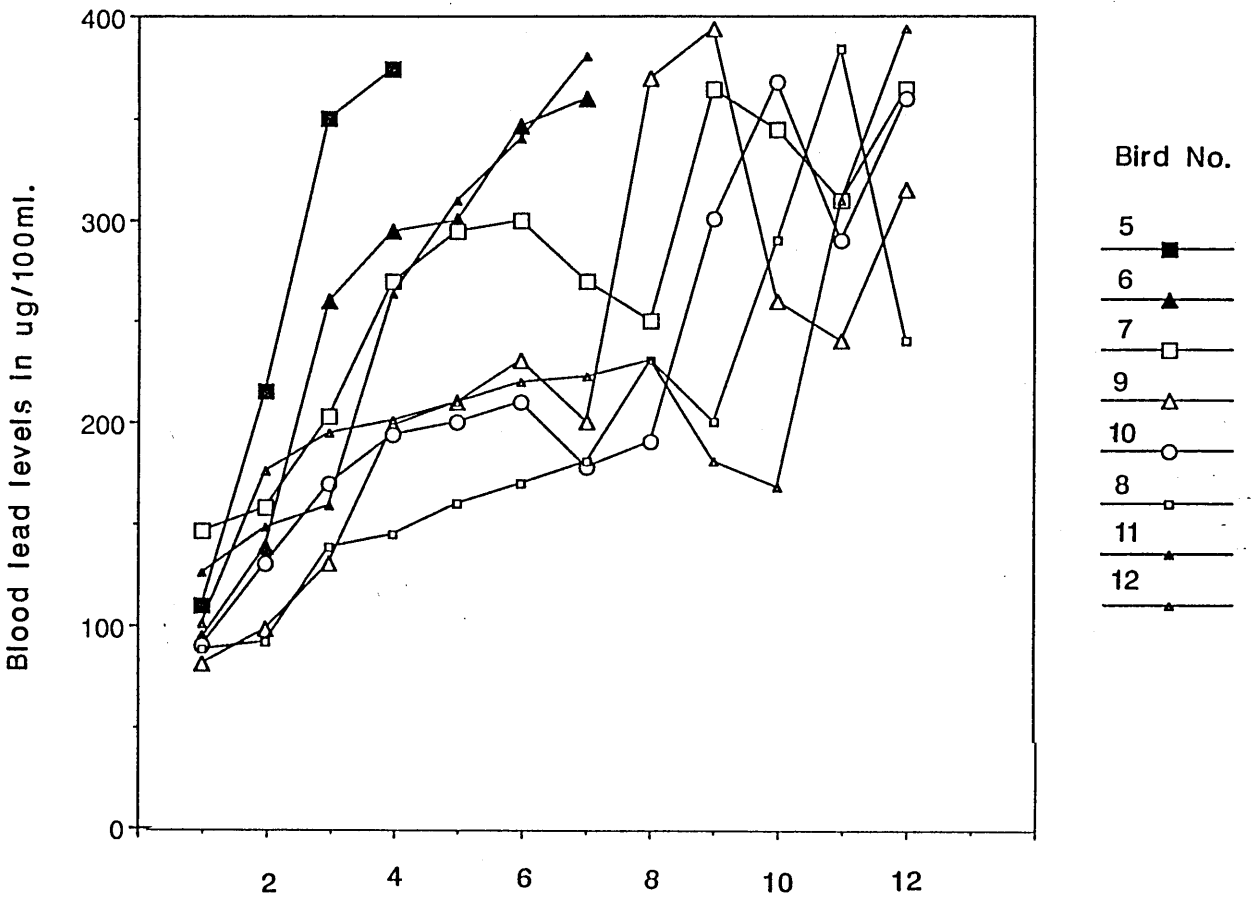
Effect of Thiamin on tissue concentration of lead (mg/kg Dry Weight) in Mallard

	Bird Number	Liver	Kidney	Bone
Control Group	1	<2	4.0	28
	2	<2	5.6	32
	3	<2	3.8	35
	4	<2	2.0	30
	mean ± SE		3.85±0.73	31.2±1.49
Lead Dosed Group	5	186	128	63.2
	6	123	193	73.6
	7	127	334	89.0
	8	146	238	137
	mean ± SE	145±14.4	223±43.2	90.7±16.31
Lead Dosed Group Thiamin Treated	9	228	560	46
	10	171	385	37
	11	140	96	9.4
	12	135	176	35.0
	mean ± SE	168±21.3	304±104.7	31.8±7.85

TABLE 2

Weight (mg) of lead administered and remaining in the gizzard at the end of the experiment

Bird Number	Original shot weight	Weight of shot eroded	Daily erosion rate (Days between dosing and death)	
5	523	93.4	(5)	18.68
6	509	108	(8)	13.5
7	523	61.9	(12)	5.16
8	520	121	(12)	10.08
9	509	258	(12)	21.5
10	512	131	(12)	10.91
11	512	89.4	(8)	11.17
12	512	259	(12)	21.58



Blood lead levels in Mallard on successive days after dosing.

Figure 1.

Chapter 7

Effects of a Diet supplemented with Sodium Phytate on the Toxicity of Lead in Mallard Ducks (*Anas platyrhynchos*)

INTRODUCTION

Recently Sears (1989) has reported that bread, when available, is the food of choice for Mute Swans, and Birkhead (1982) has shown that swans on the lower Thames rely heavily on such subsidies. It is not uncommon to see members of the public regularly feeding swans and in many areas bread forms a significant part of their diet (NCC Report 1981). Indeed, small bags of grain and bread used to be on sale for that purpose along the River Nene at Peterborough. Grain is rich in phytic acid and excessive dietary intake of phytic acid is known to suppress the availability of minerals such as iron and zinc in the gut through the formation of insoluble phytate complexes (Vohra et al. 1965). The bioavailability of some essential trace elements can also influence the absorption of lead (Calabrese 1980). Phytic acid can also bind to lead directly, thus affecting lead metabolism (Cheryan 1980, Wise & Gilburt 1981). Dietary constituents can influence the intestinal absorption of lead (Tompsett 1939). Similarly, dietary phytic acid could influence lead absorption in waterfowl which have ingested lost or discarded fishermens' lead weights.

This chapter reports (1) the effects of a diet supplemented with phytic acid on Mallard (Anas platyrhynchos) dosed with lead fishing weights. (2) the results of an experiment conducted to determine the effect of homogenates of wheat, bread and various botanical samples on solutions of lead.

MATERIALS AND METHODS

Twelve Mallard were divided into three groups each consisting of 2 males and 2 females. They were acclimatised to pen conditions for seven days before the study commenced. During this time all the birds were fed on the same diet, and grit and water was freely available throughout.

Each group was housed in a separate pen which measured 4 x 2 x 2 M high. The pens were washed each day. Two 14 litre capacity water containers were located in each pen and the water was changed twice daily. Group I, the control group, received no treatment and were fed only on the normal basal diet. Group II were force fed lead fishing weights (adjusted to a total lead weight of approximately 0.25 g) incorporated in a gelatin capsule in addition to the basal diet. Group three each received the same lead dose similarly adjusted for weight but were fed on the basal diet supplemented with sodium phytate.

On the final day of the experiment, following overnight starvation, all the birds were dosed individually with a slurry of 5 ml barium sulphate introduced directly into the proventriculous by means of a flexible plastic catheter attached to a 10 ml syringe. The experiment was terminated after 12 days.

All the birds were killed by intravenous injection of sodium pentobarbitone, after which samples of liver, kidney and bone were immediately removed for chemical analysis. Remaining lead weights were removed, washed, dried and reweighed.

Homogenate Experiment

Five gram samples of wheat, brown bread, Zostera, Spirogyra and other unidentified plant material found in the alimentary tract of swans during post-mortem examination were separately homogenised in 25 ml quantities of distilled water. Ten ml of a solution containing 500 g of lead (as lead chloride) was mixed with each sample and allowed to stand for 15 minutes. Samples of the individual supernatant solutions were then filtered and analysed for lead.

Diet

The basal diet, (both diets were specially manufactured by Birdquest International) consisted of 17% protein, 5% oil and 4% fibre, and was

supplemented with vitamins (1) and minerals (2). The experimental diet was prepared by adding 30 kg of sodium phytate to each 1000 kg of dry basal diet mixture. Both diets were manufactured to a 3 mm pellet size which is readily accepted by mallard.

		per Kg (Dry Weight)
(1)	Vitamin A	10,000 IU
	Thiamine	10 mg
	Riboflavin	12 mg
	Niacin	50 mg
	Calcium Pantothenate	22 mg
	Pyridoxine Hydrochloride	10 mg
	Vitamin B12	44 mcg
	Chloline Chloride	1020 mg
	Vitamin C	25 mg
	Vitamin D_3	3000 mg
	Vitamin E	40 mg
	Biotin	100 mcg
	Vitamin K	4 mg
	Folic Acid	2 mg
(2)	Calcium	1.0%
	Phosphorous	0.8%
	Potassium	2.0%
	Sodium	0.2%
	Iron	100 mg
	Manganese	35 mg
	Zinc	56 mg
	Copper	11 mg
	Iodine	2.1 mg
	Selenium	100 mg

RESULTS

Body weights of both of the dosed groups declined during the study: Group 2 by an average of 260 g (range 210-340 g) and Group 3 by 190 g (range 170-240 g). Mean weight loss was significantly greater in Group 2 birds than in Group 3 birds ($t = 2.63$ DF = 6 P 0.0388).

Both dosed groups showed signs of lead toxicity by their green watery droppings and soiled vent feathers. However, the group 2 birds, which received lead and the basal diet, were clinically more ill, exhibiting an unsteady gait, little interest in food and a tendency to remain in a quiet group away from disturbance. The Group 3 birds, receiving the phytate supplemented diet, did not appear unsteady when walking and could be seen to be regularly taking food and using the water bowls for bathing.

The X-rays taken 20 minutes after the slurry of barium sulphate was administered in the final day of the experiment, revealed marked peristaltic movement in the intestines of the control group of birds. Less pronounced peristaltic movement could be seen in the phytate supplemented group, but similar gut movement could not be seen in the non-supplemented lead-dosed birds. However, radio-opaque material could be clearly seen in the intestines of the non-supplemented birds, indicating a continued but abnormal gut activity.

Analysis of various tissues for lead (Table 2) showed a control (Group 1) mean level of 2, 6.4 µg g^{-1} (range 3.8-9.3 µg g^{-1}) and 18.8 µg g^{-1} (range 12.4-24.4 µg g^{-1}) in liver, kidney and bone respectively. The Group 2 birds had means of 35.9 µg g^{-1} (range 28.9-43.7 µg g^{-1}), 85.0 µg g^{-1} (range 69.7-111 µg g^{-1}) and 105 µg g^{-1} (range 84.6-132 µg g^{-1}) in their tissues, while Group 3 results were as follows, liver 17.0 µg g^{-1} (range 11.7-20.3 µg g^{-1}) kidney 34.8 µg g^{-1} (range 19.6-48.6 µg g^{-1}) and bone 72.9 µg g^{-1} (range 59.8-82.6 µg g^{-1}). In each case the Group 3 tissue levels were significantly lower than those in Group 2, liver ($t = 4.87$, DF6.0, P 0.0028), kidney ($t = 4.48$, DF6.0, P 0.0042) and bone ($t = 2.68$, DF6.0, P 0.0361).

Significantly more lead was eroded from the fishing weights in the phytate supplemented group than in the dosed group receiving only the basal diet ($t = 2.54$ DF6.0 P 0.0436) (Table 1). Daily erosion rates averaged 9.6 mg (range 6.7-12.0 mg) and 12.7 mg (range 11.8-14.3 mg) for Group 2 and Group 3 respectively. Calculation of average dietary exposure of each bird during this study based on 100 g of food per day, per bird and the relevant shot erosion, gave an equivalent dietary intake of 96 mg kg^{-1} for Group 2 birds and 127 mg kg^{-1} for Group 3 birds.

Analysis of supernatant samples from the homogenate experiments show that 80% of the lead was precipitated by wheat, 62% by bread and 2% by the remaining individual botanical specimens.

DISCUSSION

The increased erosion rate of lead in the phytate group of birds was not reflected in their body tissue values. Eroded lead, in this case, must have been made insoluble, rendering it unavailable for absorption. The diet in this programme of work was supplemented with 3% sodium phytate which was in excess of that naturally occurring in the dietary constituents.

Most seeds comprise 1-6% phytic acid (Graf 1983) and this has been shown to be capable of forming insoluble metal complexes, making them unavailable for intestinal absorption (Davies & Olphin 1979, Morris & Ellis 1980). It has also been shown that phytate alone will not precipitate lead from solution but, in the presence of calcium, a precipitate with lead is formed (Wise & Gilburt 1981). Dietary zinc can also be precipitated in this way (Likuski & Forbes 1965). Bread and wheat contain approximately 0.06 and 0.05% calcium respectively (Technical Bulletin 33). Calcium was also a major mineral constituent of the pelletised diet.

A barium test meal was administered on the final day of the experiment. The results showed that the group consuming the diet supplemented with phytate maintained a measurably more efficient gut activity than the group receiving only the basal diet. This could account for the increased erosion rate of lead seen in the sodium phytate supplemented group of birds. Also, because of continued gut activity this would also be responsible for the less marked decline in body weights seen in this group.

The difference in mean tissue values of lead caused by phytate supplementation shows that each day 8.15 mg of eroded lead was not available for absorption in this group of birds. Bearing in mind the lead eroded in the gizzards of mallard in this thesis averaged 0.011 g/lead/day the above is clearly a significant figure.

The results of the homogenate experiment show that the natural constituents of a swans diet had no detectable effect on the availability of lead in vitro. However, Jordan & Bellrose (1951) found that the most suitable food for alleviating lead poisoning was leafy green aquatic plants. These findings are in opposition to work carried out on mallard where a group of birds, given a diet with a high green food content, consumed more grit, had significantly higher liver and kidney lead residues and succumbed to lead intoxication sooner than

ducks similarly dosed but given only duck pellets to eat (French unpublished).

It is not surprising that bread is a popular food source in areas with a plentiful supply of natural vegetation (Sears 1989). Swans would need to ingest only 800 g of bread or wheat which have a protein content of approximately 9 and 12% respectively, to equal the protein content of 4 kg of wet vegetation estimated to be consumed daily by swans (Mathiasson 1973). The availability of bread usually encourages swans into many areas where lead weights are in abundance (Sears 1988, Sears 1989). The results of this experiment show that a diet supplemented with bread or wheat may be capable of altering the bioavailability of lead in swans carrying lead fishing weights in their gizzards. And furthermore, the manipulation of dietary constituents may prove to be therapeutically important in swans receiving treatment for lead poisoning.

Table 1
Weight of lead administered and remaining in the gizzard at the end of the experiment.

Bird No.	Weight of lead administered to the test group incorporated in a gelatin capsule. g	Weight of lead removed at post-mortem. g	Daily erosion rate. mg
5	0.261	0.180	6.7
6	0.264	0.120	12.0
7	0.251	0.136	9.6
8	0.260	0.138	10.2
9	0.249	0.101	12.3
10	0.250	0.098	12.6
11	0.254	0.112	11.8
12	0.259	0.087	14.3

Table 2
Results of liver, kidney and bone analysis for lead, in Mallard
Expressed in μg g^{-1} on a dry weight basis.

	Bird No.	Liver	Kidney	Bone
Group I Control (Basal diet)	1	2	8.4	18.6
	2	2	9.3	24.4
	3	2	4.1	19.8
	4	2	3.8	12.4
	Mean ± SE		6.4±1.42	18.8±2.47
Group II Lead Dosed (Basal diet)	5	39.1	84.6	112
	6	43.7	111	132
	7	28.9	74.8	91.8
	8	32.0	69.7	84.6
	Mean ± SE	35.9±3.35	85±9.19	105±10.67
Group III Lead Dosed (Basal diet with sodium phytate supplementation)	9	20.3	48.6	82.6
	10	16.8	30.0	59.8
	11	19.4	41.2	68.4
	12	11.7	19.6	80.9
	Mean ± SE	17±1.93	34.8±6.36	72.9±5.40

REFERENCES

Birkhead, M.E. (1982). Causes of mortality in the mute swan on the River Thames. J. Zool., **198**, 15-25.

Calabrese, E.J. (1980). Nutrition and Environmental Health. The influence of nutritional status on pollutant toxicity and carcinogenicity Vol II. Minerals and Macronutrients. New York: Wiley and Sons.

Cheryan, M. (1980). Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr., **13**, 297-335.

Davies, N.T. & Olphin, S.E. (1979). Studies on the phytate, zinc molar contents in diets as a determinant of Zn availability to young rats. Br. J. Nutr., **41**, 591-603.

Graf, E. (1983). Calcium binding to phytic acid. J. Agric. Food Chem., **31**, 851-855.

Jordan, J.S. & Bellrose, F.C. (1951). Lead poisoning in wild waterfowl. Biological Notes 26. Natural History Survey Division, Urbana, Illinois.

Likuski, H.J., Forbes, R.M. (1965). Mineral utilization in the rat: IV Effects of calcium and phytic acid on the utilization of dietary zinc. J. Nutr., 85, 230-234.

Mathiasson, S. (1973). A moulting population of non-breeding mute swans with special reference to flight feather moult, feeding ecology and habitat selection. Wildfowl, **24**, 43-53.

Morris, E.R. & Ellis, R. (1980). Bioavailability to rats of iron and zinc in wheat bran: response to low-phytate bran and effect of phytate/zinc molar ratio. J. Nutr., **110**, 2000-2010.

NCC Report (1981). Lead Poisoning in Swans. ISBN 0 86139 154 3.

Sears, J. (1988). Regional and seasonal variations in lead poisoning in the mute swan (Cygnus olor) in relation to the distribution of lead and lead weights in the Thames Area, England. Biol. Cons. **46**, 115-134.

Sears, J. (1989). Feeding activity and body condition of mute swans Cygnus olor in rural and urban areas of a lowland river system. Wildfowl, **40**, 88-98.

Technical Bulletin 33. Energy allowances and feeding systems for ruminants. HMSO ISBN 011 240894X.

Tompsett, S.L. (1939). The influence of certain constituents of the diet upon the absorption of lead from the alimentary tract. Biochem. J. **33**, 1237-1240.

Vohra, P., Gray, G.A. & Kratzer, F.H. (1965). Phytic acid-metal complexes. Proc. Soc. Exp. Biol. Med., **120**, 447-449.

Wise, A. & Gilburt, D.J. (1981). Binding of cadmium and lead to the calcium phytate complex in vitro. Toxicol. Lett., **9**, 45-50.

Chapter 8

Effect of Lead Ingestion on Landing Ability and Motor Coordination of Adult Pigeons (*Columba livia*)

INTRODUCTION

Changes in peripheral and central nervous system functions, the symptoms of which include tremor, fatigue, nervousness and memory loss have been attributed to lead intoxication (Valcuikas et al. 1978). Mental retardation and hyperactivity in children have also been linked to lead poisoning (Berg & Zapella 1964, Beattie et al. 1975, David et al. 1972). Workers occupationally exposed to lead showed poorer performance in behavioural test scores when compared to a control group of workers (Valcuikas et al. 1978). These tests included tasks of symbol cancellation and dexterity.

Collision with objects during flight can cause substantial mortality in swans (Ogilvie 1967). In this study over half of the swans post-mortemed had been involved in collision accidents. Swans suffering from lead poisoning have been shown to become trapped in locks and other enclosed sites and are easy to catch and handle (Simpson et al. 1979). Sublethal lead poisoning is also thought to contribute to an increased tendency to collide with objects (Birkhead &

Perrins 1986). A single injection of lead nitrate has been shown to effect the feeding behaviour of young common terns (Sterna hirundo), resulting in reduced food assimilation compared with controls (Gochfield & Burger 1988); also behavoural abnormalities have been noted in the pigeon following intubation with lead acetate (Dietz et al. 1979). Recently, O'Halloran et al. (1989) have detected abnormal levels of lead and haematological disorders in swans that have collided with objects. This present study was carried out to determine whether sublethal lead intoxication would affect the performance of pigeons in a simple performance test.

MATERIALS AND METHODS

Sixteen racing pigeons (Columba livia), ranging in age from 3 to 5 years, were divided into two groups of eight birds, four males and four females in each group. Each group was housed in a separate pen which measured 4 x 2 x 2 m. The ad libitum diet for both groups consisted of a mixture of equal quantities by weight of peas, maples peas, wheat and maize. Water and oyster shell grit were also freely available. Water bowls were provided for bathing. Prior to dosing, a blood sample was taken from each bird to determine normal blood lead values. Blood samples were then taken as far as possible every 24 hours until the

experiment was terminated after 15 days. The eight birds in the test group were, at the start, each force fed with 4 No.7 fishing weights incorporated in a gelatin capsule.

At the end of the experiment all the birds were killed by intravenous injection of sodium barbitone.

Performance test

A test was developed to assess the effect that lead might have on flying ability and landing accuracy. This test was carried out in a room measuring 3.6 x 2.4 x 3.6 m long with an east facing window 50 cm x 50 cm providing a natural light source. A sill 25 cm x 105 cm long in front of the window provided a landing platform.

The pigeons were transported to the test room altogether in a plastic chicken crate 30 x 70 x 100 cm long. The crate was covered with a sheet to exclude as much light as possible before the birds were handled for the test. Testing was carried out between 1400-1500 h each day one hour after capture. Each pigeon was removed from the crate at random. Using both hands the handler held the birds wings close to its body allowing its feet to hang freely between the fingers. The bird's head faced south away from the handler. Ten seconds after removal from the crate the bird was released from a height of 100 cm and allowed to

fall to the floor. A mat of bubble polythene measuring 100 x 100 cm was provided immediately below the bird as protection in the event of a poor landing. After release reaction was monitored and 1 minute later the bird was recaptured and placed in a separate basket away from the untested birds. The scoring system consisted of 'pass' for an accurate landing on the floor or sill, or 'fail' for an inaccurate landing. Birds which landed on the floor and then subsequently flew to the sill or sill to floor scored either pass or fail depending on the quality of the first landing. Most birds preferentially attempted landings on the sill. Collisions with the wall or sill or overbalance following landing were scored as a fail.

RESULTS

Body weights

The mean body weights were 607 g (range 580-690 g) and 597 g (range 575-640 g) at the beginning of the experiment for the control and dosed groups, and 599 g (range 585-611 g) and 588 g (range 540-610 g) at the end of the experiment. Although 3 of the dosed pigeons and 2 of the control pigeons lost weight, the group weights were not significantly different at the beginning and end of the study. Food was readily taken by both groups throughout the study.

Shot erosion and absorption

The lead shot remaining in the gizzard at the time of post-mortem was removed, washed and reweighed (Table 1). All 8 dosed birds, retained all their original dose throughout the 15-day experiment (Table 1). The mean apparent erosion was 116 mg over 15 days, or 7.74 mg per day. Calculations of average dietary exposure of each bird during our study based on 35 g of food per day per bird and the relevant shot erosion rate over 15 days, gave an equivalent dietary intake of 221 mg kg^{-1} for lead.

Post-mortem analysis

The eight dosed and eight control pigeons were examined and found to be in good general condition with no external evidence of abnormalities. The only obvious clinical sign was the production of bright green faeces in the lead-dosed group. At post-mortem, all the birds showed considerable fat reserves and no gross pathological changes were seen in the internal organs other than green staining of the gizzard lining in six of the dosed birds, but this also occurred in two of the control birds.

Blood lead levels

Twenty-four hours after dosing and throughout the period of the experiment, blood lead values were above the 40 µg/100 ml (Figure 1)

established by Birkhead (1983) as the maximum acceptable level (MAL) for swans. By day 6, median blood lead values reached a maximum of 545 µg/100 ml; thereafter values became erratic, falling to 440 µg/100 ml of blood by day 15, the end of the experiment. Blood lead levels in the experimental pigeon group were within the range of values often seen in lead poisoned swans (Simpson et al. 1979, Birkhead 1982, Sears 1988).

By day 5, when effects of the performance test could first be detected the average total exposure to lead was less than 40 mg assuming a uniform shot erosion rate. Median blood lead values at this time were 440 µg/100 ml. These levels are between 5 and 10 times higher than those in swans which were shown to be involved in collisions (O'Halloran et al. 1989).

Performance Test Results

Visual inspection of the data (Table 2) shows that the responses of the dosed birds differed from those of the control birds. All eight of the dosed birds each scored six or more failures during the performance test, whereas the eight control birds responded with at most two failures. Using Fisher's randomization test, the probability of obtaining such a result on the hypothesis of no difference in response is $\frac{2}{16C_8} = 4 \times 10^{-9}$. The pattern of the observations on the dosed

birds shows that there is a delay of 3-5 days before the effect of dosing is shown. Bellrose (1959) noted that, among dosed wild mallards, the ingestion of lead shot did not affect behaviour until after the first 5 days.

DISCUSSION

Following daily crop intubations with lead acetate solutions (72 mg/kg/day), behavioural changes in pigeons were noted (Cory-Slechta et al. 1980). Blood lead values for these birds were between 260 and 580 µg/100 ml. The changes were attributed to starvation or to a combined effect of damage to the central nervous system and starvation. As the experiment progressed, irregularities in performance were followed by regurgitation of crop fluid, crop dilatation and motor incordination. Some birds showed crop impaction with food, others lost weight. Whilst pigeons receiving 12 mg/kg/day of lead acetate by intubation exhibited no symtoms of toxicity, blood lead values for these birds were between 137-196 µg/100 ml.

Results from my study have demonstrated no pathological changes, and dosing produced no signs of overt toxicity on a calculated intake of lead of 10.5 mg/kg/day. However by day 6, median blood lead values

(545 g/100 ml) were similar to those of the pigeons receiving 72 mg/kg/day of lead acetate noted in the above experiment, and which also caused marked behavioural changes.

The two methods of dosing, intubation with lead acetate and forced feeding with lead fishing weights, may be reponsible for the different pathological changes.

Lead also affects the production of serum protein bound iodine in ducks dosed with lead shot (Goldman et al. 1977). Interference with the production of thyroid hormones, which regulate metabolic rate, enhances pituitary secretion of thyrotropin; this manifests itself in increases in thyroid weight and I^{131} uptake (Horande & Perez-Castrillo 1961). This compensatory mechanism eventually becomes exhausted and a state of hypothyroidism ensures (Sandstead 1967).

Change in thyroid activity resulting in a hypothyroidal state has been shown to severely depress metabolic rate (Sturkie 1965). Interference with thyroid activity by other environmental contaminants has also been noted (Jefferies & French 1971). In this particular study, low doses of DDT caused birds to exhibit a hypothyroidal state and high doses caused a hyperthyroidal state. This phenomena could also occur with other toxic chemicals including lead, resulting in either over- or under-activity.

Levels of lead similar to those found in the kidney have been demonstrated in the pancreas of mallard dosed with gunshot (Longcore et al. 1974), and in swans; increased lead levels in the pancreas have been shown to be due to the ingestion of fishing weights (O'Halloran et al. 1988). Whether these levels of lead in the pancreas cause sublethal effects is not known. However, one of the metabolic activities of the mammalian pancreas is to control glucose metabolism via its secretion of the hormone, insulin. O'Halloran et al. (1988) also noted changes in circulating plasma glucose levels in swans suffering from acute lead poisoning. Reduced blood glucose levels may be the result of starvation, often seen in lead-poisoned swans due to impaction of the alimentary tract, or a direct effect on pancreatic activity. Either way, any sustained change in, or interference with, the regulation of metabolic rate would seriously impare the ability to cope with an urgent call on energy and could also therefore increase risks of colliding with objects.

Ducks, whose diet has been dosed with cadmium, ran twice as far from a fright stimulus as did controls or birds receiving a higher dose (Heinz & Haseltine 1983). This type of reaction has also been noted in quail receiving endrin (Krietzer 1980). Also ducks receiving methyl mercury were hyper-responsive in avoidance behaviour (Heinz 1979). Ducklings dosed with lead also showed lower scores in open field behaviour tests

when compared with controls, but the difference was not significant (Frederick 1976). Hypo- or hyper-responsiveness could be just as damaging in avoidance behaviour and could manifest itself in increased collision with objects. Tests carried out on a group of workers occupationally exposed to lead and a group of controls showed significant differences in performance when measured by the Critical Flicker Frequency Test (Betta 1983). This test is capable of measuring vigilance and reactivity, two important factors in avoidance response. Similarly workers exposed to lead scored significantly lower marks during performance tests than did controls (Valcuikas et al. 1978).

In the latter two studies, the blood lead levels which were needed to affect performance, in the test workers were considerably lower than values seen in lead burdened swans (Simpson et al. 1979, Birkhead 1982, Sears 1988, O'Halloran et al. 1989).

Also Mallard carrying a single lead shot in their gizzard were affected in such a way as to make them more vulnerable to hunters than lead-free ducks (Bellrose 1959). As the number of lead shot in their gizzards increased so did their vulnerability. In this same study weakness and fatigue caused by lead poisoning reduced the ability of wild mallard to migrate. The larger the number of shot ingested, the greater the reduction in movement.

With our present knowledge, it is impossible to hypothesize what combination of factors cause the tendency of lead burdened swans to collide with overhead cables. But the changes in physiology due to lead already mentioned have all been shown to affect normal performance in one way or another.

The results of this experiment have shown that sublethal lead intoxication is capable of affecting a birds' motor coordination and landing ability. Furthermore, these affects were detected when blood lead values, in the experimental birds, were within the range of those seen in live wild swans.

REFERENCES

Beattie, A.D., Morre, M.R. & Goldberg, A. (1975). Role of chronic low-level lead exposure in the etiology of mental retardation. Lancet **1**, 589.

Bellrose, F.C. (1959). Lead poisoning as a mortality factor in waterfowl populations. Ill. Nat. Hist. Surv. Bull. **27**: 235-287.

Berg, J.M. & Zapella, M. (1964). Lead poisoning in childhood with particular reference to pica and mental sequelae. J. Ment. Detic. Res., 8, 44-53 (1964).

Betta, A., DeSanta, A., Savoritto, C. & D'Andrea, F. (1983). Flicker Fusion Test and Occupational Toxicology: Performance Evaluation in Workers Exposed to lead and solvents. Human Toxicol., 2, 83-90.

Birkhead, M. (1982). Causes of mortality in the mute swan Cygnus olor on the River Thames. J. Zool. Lond. 198: 15-20.

Birkhead, M. (1983). Lead levels in the blood of mute swans Cygnus olor on the River Thames. J. Zool. Lond. 199, 59-73.

Birkhead, M. & Perrins, C. (1986). The Mute Swan. London: Croom Helm.

Bryce-Smith, D., Mathews, J. & Stephens, R. (1978). Mental Health effect of lead on children. Ambio 7, 192.

Clarke, E.G. & Clarke, M.L. (1975). Veterinary Toxicology. London: Bailiiere.

Cory-Slechta, D.A., Garman, R.H. & Sadman, D. (1980). Lead-induced crop dysfunction in the pigeon. Toxicol. Appl. Pharmacol., **52**, 462-467.

David, D., Clark, J. & Voeller, K. (1972). Lead and hyperactivity. Lancet **2**, 900-903.

Dieter, M.P. & Finley, M.T. (1979). Delta-aminolevulinic acid dehydratase enzyme activity in blood, brain and liver of lead-dosed ducks. Environ. Res. **19**: 127.

Frederick, R.B. (1976). Effects of lead nitrate ingestion on open-field behaviour of mallard ducklings. Bull. Environ. Contam. Toxicol., **16**, 6 739-742.

Gochfield, M. & Burger, J. (1988). Effects of lead on growth and feeding behaviour of young common terns (Sterna hirundo). Arch. Environ. Contam. Toxicol. **17**, 513-517.

Goldman, M., Dillon, R.D. & Wilson, R.M. (1977). Thyroid function in Peking ducklings as a consequence of erosion of ingested lead shot. Toxicol. and Appl. Pharmacol. **40**, 241-246.

Gray, L.E. & Reiter, L. (1977). Lead-induced developmental and behavioural changes in the mouse. Toxicol. Appl. Pharmacol. **41**, 140.

Heinz, G. (1975). Effects of methylmercury on approach and avoidance behaviour of mallard ducklings. Bull. Environ. Contam. Toxicol. **13**, 554-564.

Heinz, G.H. (1979). Methylmercury: Reproductive and behavoural effects on three generations of mallard ducks. J. Wildl. Manage. **43**, 394-401.

Heinz, G.H. & Haseltine, S.D. (1983). Altered avoidance behaviour of young black ducks fed cadmium. Experimental Toxicology and Chemistry **2**, 419-421.

Hirano, A. & Kochen, J.A. (1973). Neurotoxic effects of lead in the chick embryo. Lab. Investigations **29**, 659-668.

Horande, M. & Perez-Castrillo, R. (1961). Influence of subacute lead poisoning on 1^{131} uptake by the rat thyroid. Ann. Endocrinol. **22**, 898-901.

Jefferies, D.J. & French, M.C. (1971). Hyper- and hypo-thyroidism in pigeons fed DDT: an explanation for the "thin eggshell phenomenon". Environ. Pollut., **1 (3)** 235-242.

Krietzer, J.F. (1980). Effects of toxaphene and endrin at very low dietary concentrations on discrimination aquisition and reversal in bobwhite quail Colinus virginianus. Environ. Pollut. **23A,** 217-230.

Ogilvie, M.A. (1967). Population changes and mortality of the mute swan in Britain. Wildfowl Trust Ann. Rep. **18:** 64- .

O'Halloran, J., Myers, A.A. & Duggan, P.F. (1989). Some sub-lethal effects of lead on mute swan Cygnus olor. J. Zool. London **218,** 627-632.

Sandstead, H.H. (1967). Effect of chronic lead intoxication on in vitro I^{131} uptake by the rat thyroid. Proc. Soc. Exp. Biol. Med. **124,** 18-20.

Sears, J. (1988). Regional and seasonal variations in lead poisoning in the mute swan (Cygnus olor) in relation to the distribution of lead and lead weights in the Thames Area, England. Biol. Cons., **46,** 115-134.

Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of mute swans. Environ. Pollut., **18**, 187-202.

Sturkie, P.D. (1965). Avian Physiology. London: Bailliere. Tindall & Cassell.

Valcuikas, J.A., Lillis, R., Eisinger, J., Blumberg, W.E., Fishchbein & Selikoff, I.J. (1978). Behavoural indicators of lead neurotoxicity: Results of a clinical field survey. Int. Arch. Occup. Environ. Hlth. **41**, 217-236.

TABLE 1

Weight of lead shot administered and remaining in the gizzard of pigeons at the end of the experiment (15 days)

Bird No.	Weight of four No.7 shot administered to the test group incorporated in a gelatin capsule g	Weight of lead shot removed at post-mortem. Number of shots is in parenthesis g
9	0.307	0.183 (4)
10	0.316	0.238 (4)
11	0.320	0.193 (4)
12	0.300	0.206 (4)
13	0.360	0.229 (4)
14	0.334	0.223 (4)
15	0.329	0.191 (4)
16	0.310	0.184 (4)

TABLE 2

Performance Test Results

Days after dosing with lead shot
(P = Pass, F = Fail)

	Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	1	P	P	P	P	P	P	P	P	F	P	P	P	P	P	P
	2	P	F	P	P	F	P	P	P	P	P	P	P	P	P	P
	3	P	P	P	P	P	P	P	F	P	P	P	P	P	P	P
	4	P	P	F	F	P	P	P	P	P	P	P	P	P	P	P
	5	P	P	P	P	F	P	P	P	P	P	P	P	P	P	P
	6	P	P	P	P	P	P	P	P	F	F	P	P	P	P	P
	7	P	P	F	P	P	P	P	P	P	P	P	P	P	P	P
	8	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Total P		8	7	6	7	6	8	8	7	6	7	8	8	8	8	8
Lead Dosed	9	P	P	F	F	F	P	F	P	P	F	F	F	F	F	F
	10	P	F	P	P	P	F	P	F	F	P	P	F	F	P	P
	11	P	P	F	P	P	F	F	F	P	P	P	F	F	F	P
	12	P	P	P	F	F	P	F	P	F	P	F	P	P	F	F
	13	P	P	P	F	F	F	P	F	F	F	P	F	F	P	P
	14	P	P	F	P	P	F	F	F	F	F	F	F	P	F	F
	15	P	P	P	F	F	F	F	P	F	F	P	P	F	F	F
	16	P	P	P	P	P	F	F	F	P	F	F	F	F	P	F
Total P		8	7	5	4	4	2	2	2	2	3	3	2	2	3	3

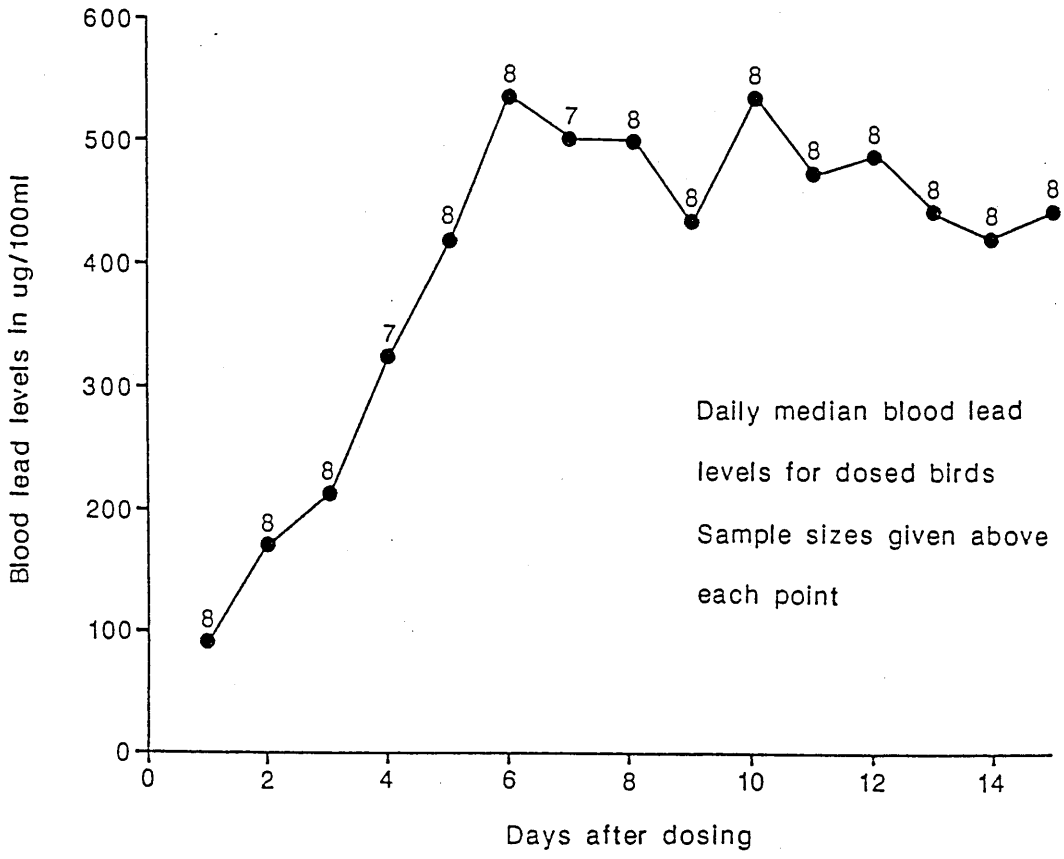

Blood lead levels in ug/100ml
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
Days after dosing
8
8
8
7
8
8
7
8
8
8
8
8
8
8
Daily median blood lead
levels for dosed birds
Sample sizes given above
each point

Fig 1

Chapter 9

Investigation into the Effects of Ingestion of Zinc Shot by Mallard Ducks (*Anas platyrhynchos*)

INTRODUCTION

The poisoning of wildfowl following the ingestion of spent lead gunshot has been well researched, particularly in the USA (Jordan & Belrose, 1951; Belrose, 1959; Trainer & Hunt, 1965; US Fish and Wildlife Service, 1976). The problem of lead poisoning of wildfowl has been demonstrated in Italy (Del Bono, 1970), Denmark (Clausen et al., 1975) and Great Britain (Beer & Stanley, 1963-64; Mudge, 1983).

In 1979, a second lead poisoning threat to swans was identified as discarded lead fishing weights (Simpson et al., 1979). Surveys carried out in the East Midlands (Hunt, 1980) and on the River Thames (Birkhead, 1981) showed that up to 80% of deaths of mute swan (Cygnus olor) in certain areas were due to lead poisoning, following the ingestion of lead fishing weights. The same problem has been recorded in whooper (Cygnus cygnus) and Bewick swans (Cygnus bewickii) (Owen & Cadbury, 1975; French, 1982). A summary of the available information on swans was published by the Nature Conservancy Council (1981). These findings, coupled with the recommendation of the Royal Commission on

Environmental Pollution, gave impetus to the search for alternatives to lead, both as gunshot and as fishing weights. This project is concerned with testing the safety of zinc shot provided by the manufacturer AM & S Europe.

MATERIALS AND METHODS

The birds tested were 1-year-old mallards (Anas platyrhynchos) reared by a registered wildfowl dealer. Thirty-eight birds were used in the study, divided into two separate experiments of different dosage levels and carried out at different times. All birds were acclimatised to pen conditions for 14 days before the studies commenced. The birds had free access to food, grit and water at all times during the experiment. The food consisted of wheat, barley and turkey crumbs, a mixture found suitable for ducks on previous occasions. The lower dose experiment consisted of three groups of ten birds, five males and five females in each group. The high dose study consisted of eight birds divided into two groups, two males and two females in each.

Each group was housed in a separate pen which measured 4 x 2 x 2 m. The pens were washed each day. Two 14-litre capacity water containers were located in each pen and were changed twice daily.

Each bird was weighed and radiographed before dosing. The lower dose test birds (five males and five females) were each given a single oral dose of five No.6 high grade zinc shot incorporated in gelatin capsules. The control group of five pairs each received empty gelatin capsules. The remaining five pairs did not receive any treatment and were used as 'super-controls' to highlight any possible effects of gelatin ingestion or X-ray exposure in the other birds.

The high dose test birds (two males and two females) were each dosed orally with ten No.6 high grade zinc shot incorporated in gelatin capsules. The control group again received empty capsules. All shot used in this investigation was produced from zinc of 99.9% purity.

Samples of liver, kidney and feathers, which were removed at the end of the study, were oven dried at 80°C for 24 h and then digested in 10 ml of concentrated nitric acid for 18 h at room temperature, followed by boiling for 1 h. The extract was cooled and diluted to a volume of 25 ml with glass-distilled water. Final chemical analyses were carried out using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

After 2 days, all the birds in the dosed and control groups (but not

the super-control group) were X-rayed to ascertain whether the shot was retained in the gizzard. All the lower dosed birds had retained their original dose, except one bird which had voided one zinc shot which was discovered in the flight pen. Only one of the high dose birds had retained all of the original shot. The other three, two females and one male, had voided four, four, and two, respectively, of the original dose. Five shots were recovered from the pen and the remaining five were assumed to have been washed out in the daily cleaning process. The X-ray procedure was repeated after 7 and 14 days, and no further loss of shot was observed. Both experiments were terminated after 28 days. All the birds survived the study with no visible ill-effects of dosing. Moreover, it was noted that all the zinc dosed birds had markedly better conditioned plumage than did the controls and super-controls, even though they had not moulted.

The ten dosed and ten control mallards from the lower dose experiments were examined and found to be in good general condition with no macroscopical evidence of lesions or abnormalities, other than some abrasions to the feet caused by the concrete flight floors. The birds were killed by intravenous injection of sodium pentobarbitone and immediately post-mortemed. One control bird showed marked feather loss and small internal fat reserves, but new feathers were appearing and no gross pathological changes were seen in the internal organs. Liver and

kidney samples were removed for histo-pathological examination, which supported the gross post-mortem findings that there were no important differences between birds in the dosed and control groups.

The high dosed birds were similarly treated, but no histo-pathology was carried out in that study.

Body weights

The three groups in the lower dose experiment each gained an average of 12% weight over 28 days. Mean weight gains for the super-controls were 94 g (range 88 to 106 g), controls 101 g (range 94 to 137 g) and five shot dose 97 g (range 84 to 103 g). These differences were not statistically significant.

The two groups in the high dose experiment each gained an average of 2% weight. Mean weight gains for the controls were 16 g (range 14 to 18 g), and 10 shot dose 19 g (range 13 to 20 g), again not significantly different.

Colder environmental conditions prevailing at the time of the second study may have influenced body condition.

Low dose study: Daily maximum 20.7°C, Minimum 11.6°C, Mean 16.1°C.
High dose study: Daily maximum 4.0°C, Minimum -3.7°C, Mean 0.1°C.

Effect on iron, copper and zinc levels in tissues

In their study Grandy et al. (1968) measured iron levels in the liver in order to determine the effect of zinc intoxication, and also to assess the degree of haemosiderosis which they found in the test group and also some of the control ducks. We measured iron and also copper levels in the liver and kidney, since it is known that anaemia, which was noted by Gasaway & Buss (1972), is a common expression of copper deficiency.

Chemical analyses of liver, kidney and feathers were carried out on tissue removed at the time of post-mortem from both experiments. The results of iron and copper determination in the livers and kidneys of all the dosed and control birds are shown in Table 1. Liver iron levels in the lower dose birds (mean 2109 $\mu g\ g^{-1}$; range 1385-4081 $\mu g\ g^{-1}$) did not exceed those of the control birds (mean 2909 $\mu g\ g^{-1}$; range 1274-11300 $\mu g\ g^{-1}$). The high dose experiment reflected the same liver iron pattern (mean 1906 $\mu g\ g^{-1}$; range 1342-2964 $\mu g\ g^{-1}$) for the dosed birds, compared to the control group mean of 1968 $\mu g\ g^{-1}$;(range 1268-3100 $\mu g\ g^{-1}$).

Liver and kidney copper levels reflected a normal pattern in both dosed groups, except control bird No.6 which had a kidney copper level of 492 $\mu g\ g^{-1}$, a figure I cannot explain.

TABLE 1
Results of Liver and Kidney Analyses for Copper and Iron of Mallard Expressed in $\mu g\ g^{-1}$ on a Dry Weight Basis

	Bird No.	Liver		Kidney	
		Copper	Iron	Copper	Iron
Control	1	414	11300	27.5	794
	2	205	1640	26.3	802
	3	263	1274	25.0	711
	4	476	1979	15.1	577
	5	291	2157	25.0	1019
	6	258	3399	492.0	692
	7	742	1545	18.5	629
	8	216	1310	19.7	530
	9	392	3015	14.7	772
	10	127	1473	19.2	551
	Mean ± SE	338±56	2909±959	68.3±47	707±47
Low dose	11	380	1630	16.4	500
	12	255	4081	35.7	571
	13	339	2321	22.7	621
	14	360	2720	30.0	600
	15	263	1412	31.2	775
	16	311	2551	23.2	625
	17	352	1760	27.0	345
	18	310	1805	18.0	666
	19	377	1428	28.5	585
	20	310	1385	28.1	687
	Mean ± SE	325±14	2109±267	26.0±1.9	597±36
Control	21	374	1268	26.2	714
	22	339	3100	27.1	528
	23	700	1814	19.8	552
	24	422	1692	20.0	800
	Mean ± SE	458±82	1968±394	23.2±2	648±65
High dose	25	397	1772	21.6	791
	26	682	2964	28.4	718
	27	416	1342	25.1	530
	28	320	1548	18.9	511
	Mean ± SE	453±79	1906±363	23.5±2	637±69

TABLE 2
Results of Liver, Kidney and Feather Analyses of Mallard for Zinc on a Dry Weight Basis $\mu g\ g^{-1}$.

	Bird No.	Liver	Kidney	Feather
Control	1	196	57.6	125
	2	164	65.8	136
	3	182	76.9	135
	4	247	54.4	138
	5	171	67.3	118
	6	162	76.9	144
	7	204	48.3	101
	8	112	60.6	107
	9	162	45.5	133
	10	116	57.7	150
	Mean ± SE	171±13	61.1±3.4	128±5
Low dose	11	222	50.0	104
	12	260	95.2	130
	13	208	83.3	125
	14	209	90.1	144
	15	180	68.7	125
	16	204	80.3	140
	17	270	88.5	153
	18	194	69.4	105
	19	214	85.7	134
	20	216	78.1	100
	Mean ± SE	217±9	78.9±4.2	126±5.7
Control	21	201	54.1	153
	22	196	86.2	146
	23	180	48.3	151
	24	112	69.0	130
	Mean ± SE	172±21	64.4±8.5	145±5.2
High dose	25	180	61.0	134
	26	190	80.6	159
	27	246	95.1	141
	28	230	51.2	140
	Mean ± SE	211±16	71.9±9.8	143±5.4

TABLE 3
Weights of Zinc Shot Administered and Remaining in the Gizzard at the End of the Experiments

Weight of five No.6 shot administered to test group incorporated in a gelatin capsule.		Weight of zinc shot removed at post-mortem. Number of shots is in parentheses.
Bird No.	g	g
11	0.388	0.127 (5)
12	0.401	0.101 (4)
13	0.405	0.184 (5)
14	0.396	0.132 (5)
15	0.394	0.120 (5)
16	0.387	0.151 (5)
17	0.395	0.130 (5)
18	0.398	0.136 (5)
19	0.407	0.147 (5)
20	0.404	0.141 (5)
25	0.804	0.139 (6)
26	0.792	0.162 (6)
27	0.774	0.372 (10)
28	0.801	0.286 (8)

Analyses of the livers for zinc (Table 2) showed a control mean level of 171 µg g^{-1} (range 112-247 µg g^{-1}) and a low dose mean level of 217 µg g^{-1} (range 180-270 µg g^{-1}). Only two birds from either dosed group had liver zinc levels (260 and 270 µg g^{-1}) which exceeded the maximum control level of 247 µg g^{-1} zinc. However, the low dosed group had statistically higher levels of zinc in the liver ($t = 3.0$, DF 18, P 0.01), compared with the controls.

Feather levels of zinc were not statistically different between the groups. The birds were in full feather when the experiment began, and zinc deposition in the feathers of moulting birds was not studied.

Shot erosion and absorption

The zinc shot remaining in the gizzard at the time of post-mortem was removed, washed and reweighed.

Of the 14 dosed birds from both groups, 10 retained all their original dose throughout the 28-day experiment (Table 3). The mean apparent erosion rate for the lower dose group was 261 mg over 28 days, or 9.32 mg per day, and for the high dose group (based on one bird which retained all 10 shot) it was 402 mg, or 14.4 mg per day.

A survey carried out in the USA by Belrose (1959) showed that, of the duck gizzards which contained lead shot, 65% contained one shot and 15%

contained two. Similar figures for mallard gizzards have been given by Mudge (1983) from a survey carried out in Great Britain. The lower dose (five shot) in the present study therefore represents a realistic maximum which could be expected under field conditions.

Calculations of average dietary exposure of each bird during this study based on 100 g of food per day, per bird and the relevant daily shot erosion rate over 28 days, gave an equivalent dietary intake of less than 100 mg kg^{-1} of zinc for the low dose birds, and 150 mg kg^{-1} for the high dose birds. The latter calculation was again based on the one bird which retained all ten shot, and which was the most testing case from this study.

It is known that developing chickens can consume feed contaminated with various zinc salts up to a level of 1000 mg kg^{-1} for nine weeks without harm (Mehring et al., 1956; Robertson & Schaible, 1960; Johnson et al., 1962). To exceed these levels of zinc intake in our study it would have required in excess of 50 shot per bird to be ingested.

Gasaway & Buss (1972) studied the effects on mallard of even higher levels of zinc exposure. They blended the chemical zinc carbonate into pelleted bird feed at concentrations ranging from 3000 mg kg^{-1} to 12000 mg kg^{-1}. It is not clear whether the mallard's subsequent severe

loss of appetite, weight and locomotion, plus anaemia, etc., were due to the unpalatability of the chemical, or to toxic effects (Wobeser, 1981). Either way, it is obviously undesirable for birds to have to absorb these extremely high levels of zinc compound in their food.

At the other end of the spectrum, zinc is required as a trace element in the diet of animals and is often added to feeds in concentrations of around 250 mg kg^{-1}. The current study sought to relate the intake by mallards of realistically high numbers of metallic zinc shot (as found in normal field conditions) to measured rates of absorption, and subsequent effects on health. It has been demonstrated that a bird with 10 shot in its gizzard effectively absorbs the equivalent of 150 µg g^{-1} zinc in its diet in 28 days. The most severe zinc toxicity studies (Chupp & Dalke, 1964; Gasaway & Buss; 1972) cannot therefore be related to the likely rates of zinc shot ingestion by wildfowl. They may be more relevant to the specific effects of mining and smelting pollution on these birds.

In a similar study to ours Grandy et al. (1968) reported that three out of fifteen mallards dosed with eight No.6 zinc-based shot died within 30 days of ingestion. Only three surviving birds retained some shot until the end of the 30-day study. Severe weight losses, plus walking and muscular control problems were noted. The results contrast

strongly with ours, possibly because Grandy et al. used shot produced from a material containing only 92% zinc and 7% undetermined impurities.

CONCLUSION

It is established that, each year in Great Britain, 8000 mallards die after ingesting lead gunshot (Mudge, 1983) and nearly 4000 mute swans die after accidentally picking up lead fishing weights (NCC, 1981). In the USA, a programme of usage of non-toxic shot has improved the situation, and now non-toxic pellet areas have been established in 32 States (White & Stendell, 1977). In Great Britain new laws (The Control of Pollution (Anglers' Lead Weights) Regulations, 1986) were introduced at the beginning of 1987, to help protect the indigenous swan population. It is now an offence to import or supply lead weights for angling of over 0.06 g and up to and including 28.35 g. There is some reason to anticipate a similar movement concerning the usage of lead based gun shot, now that the former issue has been resolved.

The zinc shot prepared by AM & S Europe for this experiment has shown itself to be non-toxic, based on observations of the mallard during the 28-day experimental period, post-mortem examination, histo-pathological

examination and also chemical analysis of various tissues removed at post-mortem. Furthermore, the rates of zinc absorption have been measured and found to be considerably lower than the levels often fed to farm animals in the form of beneficial food supplements. Based on the data currently available, it would seem that zinc, in metallic form, would be an acceptable material to use as a base in fishing weights and shotgun pellets.

REFERENCES

Beer, J.V. & Stanley, P. (1963-4). Lead poisoning in the Slimbridge Wildfowl Collection. Ann. Rep. Wildfowl Trust, **16**, 30-4.

Belrose, F.C. (1959). Lead poisoning as a mortality factor in waterfowl populations. Bull. Ill. St. Nat. Hist. Surv., **27**, 235-88.

Birkhead, M. (1981). How the fishermen kill the swans. New Scient., **90**, 14-15.

Chupp, B.R. & Dalke, D.P. (1964). Waterfowl mortality in the Coeur d'Alene River Valley, Idaho. J. Wildl. Mgnt., 28(4), 672-702.

Clausen, A.B., Dalsgaard, H. & Woldstrup, C. (1975). Mabrud of blyforgiftning blandt danske knopsuaner (Cygnus olor). Dansk. Vet. Tidssker., **21**, 843-7.

Del Bono, G. (1970). Il saturismo degli uccelis aquatici arrali. Fac. Med. Vet. Univ. Pisa, **23**, 102-51.

French, M.C. (1982). Lead poisoning in Bewick swans. BTO News, **121**, 1.

Gasaway, W.C. & Buss, I.O. (1972). Zinc toxicity in the mallard duck. J. Wildl. Mgnt., **36(4)**, 1107-17.

Grandy, J.W., Locke, L.N. & Begley, G.E. (1968). Relative toxicity of lead and five proposed substitute shot types to pen-reared mallards. J. Wildl. Mgnt., **32(3)**, 483-8.

Hunt, A.E. (1980). Mute swan investigations - lead poisoning. BTO News, **110**, 1-2.

Johnson, D.Jr., Mehring, A.L.Jr., Saving, F.X. & Titus, H.W. (1962). The tolerance of growing chickens for dietary zinc. Poultry Sci., **41(1)**, 311-17.

Jordan, J.S. & Belrose, F.C. (1951). Lead poisoning in wild waterfowl. Ill. St. Nat. Hist. Surv. Biol. Notes, No.26.

Mehring, A.L.Jr., Brumbough, J.H. & Titus, H.W. (1956). A comparison of the growth of chicks fed diets containing different quantities of zinc. Poultry Sci., **35**, 956-8.

Mudge, G.P. (1983). The incidence and significance of ingested lead pellet poisoning in British wildfowl, Biological Conservation, **27**, 333-72.

Nature Conservancy Council (NCC) (1981). Lead poisoning in swans. Report of the NCC's Working Group, London, NCC.

Owen, M. & Cadbury, C.J. (1975). The ecology and mortality of swans at the Ouse Washes, England. Wildfowl, **26**, 31-42.

Robertson, R.H. & Schaible, R.J. (1960). The tolerance of growing chicks for high level of different forms of zinc. Poultry Sci., **39(4)**, 893-6.

Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of mute swans. Environ. Pollut., **18**, 187-202.

Trainer, D.O. & Hunt, R.A. (1965). Lead poisoning of whistling swans in Wisconsin. Avian Dis., 9, 252-64.

US Fish and Wildlife Service (1976). Proposed use of steel shot for hunting waterfowl in the United States. Final Environmental statement, Washington, DC, US Dept. of the Interior, Fish and Wildlife Service.

White, D.H. & Stendell, R.C. (1977). Waterfowl exposure to lead and steel on selected hunting areas. J. Wildl. Mgnt., **41**, 496-75.

Wobeser, G.A. (1981). Diseases of wild waterfowl. Plenum Press, New York.

Chapter 10

Modification of Lead Toxicity following Simultaneous Ingestion of Lead and Zinc by Mallard Ducks (*Anas platyrhynchos*)

INTRODUCTION

The toxicity of environmental pollutants is often attributed to single elements or compounds, when in fact an interaction between two or more contaminants could have a profound effect on the physiological state of the animal (Durham 1967). Some combinations can potentiate the toxicity of individual chemicals (Keplinger and Deichman 1967; Dieter and Ludke 1978), while other combinations can yield some degree of protection (Ball et al. 1954; Cerklewski and Forbes 1976).

It is known that dietary balance (Barltrop and Khoo 1975), physiological condition (Finley and Dieter 1978), age (Forbes and Reina 1972), dietary constituents (Tompsett 1939), seasonal factors (McEwen 1963) and temperature (Rattner et al. 1987) can influence the degree of toxicity of environmental contaminants.

This study investigates the possible effect of the ingestion of lead fishing weights which are known to be widely available to our indigenous swan population, and a proposed fishing weight

substitute, zinc, to mallard (Anas platyrhynchos), a situation which could readily occur in regularly hunted or fished areas.

METHODS

Eighteen adult mallard (Anas platyrhynchos) were used in this study, divided into three separate groups of six birds, three males and three females in each group. The birds were acclimatised to pen conditions for nine days prior to the study. The birds had free access to food, grit and water at all times during the trial period. The food consisted of wheat, barley and turkey crumbs in equal volumes, a mixture found to be suitable for ducks on previous occasions. Each group was housed in a separate flight pen which measured 4 x 2 x 2 metres. The pens were washed each day. Two 14-litre water containers were located in each pen and changed twice daily.

Each bird was radiographed and weighed before dosing. The lead dosed birds, group two, were each given a single oral dose of five lead fishing weights incorporated in a gelatin capsule. The combined lead and zinc dosed birds, group three, were each given a single oral dose of five lead fishing weights and five No.6 high grade zinc shot incorporated in a gelatin capsule. The control group birds, group one, each received an empty gelatin capsule.

The lead weights and the zinc shot were weighed before dosing commenced.

X-rays were taken after 4 days to determine if any weights had been lost from the gizzard. Ten days after dosing the activity of the gizzard and alimentary tract in all the birds was determined by means of a barium test meal. The birds were dosed after overnight starvation with a slurry of barium sulphate introduced directly into the proventriculus by means of plastic catheter attached to a 10 ml syringe. X-rays were then taken 20 minutes, 40 minutes and 1 hour after dosing.

RESULTS AND DISCUSSION

After four days two birds, one from each group, both females, number 11 and 16, had voided one shot each. At post-mortem the lost shot from birds 11 and 16 was shown to be lead. All the dosed birds showed signs of lead poisoning, green stained vent feathers, lack of appetite and consequent loss of weight. The clinical signs of lead poisoning were more apparent in the group II birds than in group III, the combined dosed birds. In the second week of the study all the dosed birds showed signs of weakness and a desire to congregate in groups away from

disturbances. The birds were killed at the end of the study by intravenous injection of sodium pentobarbitone and immediately post-mortemed. The control group were in good condition, with large body fat reserves, and showed no signs of disease or damage. The birds from both dosed groups showed typical signs of lead intoxication, distended gall bladders, severe loss of weight and dark and flabby intestines.

Four birds from group 2, 2 males and 2 females had impacted gizzards, the remaining female had a substantial quantity of food in the gizzard but none in the alimentary tract, the digestive system of the remaining male had a small quantity of food present but was flabby and dark in colour. However, none of the lead-zinc dosed group of birds showed any impaction of food in the gizzard or proventriculus.

Body weights

All the control birds gained weight, and mean weight gains were 88 g (range 80-111 g). Mean weight losses for the lead dosed group and combined lead-zinc dosed group were 349 g (range 310-394 g) and 286 g (range 237-310 g) respectively. The lead dosed group lost significantly more weight than did the combined lead-zinc dosed group ($t = 3.37$, df 10, P 0.0071).

Shot erosion

The zinc and lead weights remaining in the gizzard at the time of post-mortem were removed, washed and reweighed.

Of the twelve dosed birds from both groups, ten retained all their original dose throughout the 18-day experiment (Table 2). The mean rate of erosion of lead for group 2, based on the five birds which retained all the original lead dose, was 206 mg over 18 days or 11.4 mg per day. The total erosion and daily exposure of lead in group 3 was significantly higher than group 2 ($t = 3.01$, df 8.0, P 0.0168) at 219 mg over the 18 days trial or 12.2 mg per day again based on the five birds which retained all the original dose. The mean erosion rate of zinc in group 3 was 118 mg over 18 days or 9.9 mg per day.

Analysis of tissue for lead and zinc

Chemical analyses of liver, kidney and bones were carried out on tissue removed at the time of post-mortem from all three groups of birds. The results of lead and zinc determinations in the various tissues are shown in Table 1. Liver lead levels in group 2 (mean 153 $\mu g\ g^{-1}$, range 70.5-216 $\mu g\ g^{-1}$) were exceeded by those in group 3 (mean 539 $\mu g\ g^{-1}$ range 312-1180 $\mu g\ g^{-1}$) and were statistically different ($t = 2.91$, df 10, P 0.0155). The maximum liver value for lead, 216 $\mu g\ g^{-1}$ in group 2, was lower than the minimum liver value for lead, 312 $\mu g\ g^{-1}$, in

group 3. Kidney lead residues showed a similar pattern: group 2 mean 266 µg g^{-1} (range 398-344 µg g^{-1}) and group 3 mean 447 µg g^{-1} (range 398-521 µg g^{-1}) ($t = 4.05$, df 10, P 0.0023). The maximum value of group 2, 344 µg g^{-1}, did not exceed the minimum value of group 3, 398 µg g^{-1}.

Bone levels showed a reverse pattern: mean bone levels for group 2 and group 3 were 299 µg g^{-1} (range 226-356 µg g^{-1}) and 110 µg g^{-1} (range 68.2-140 µg g^{-1}) respectively and were statistically different ($t = 7.18$, df 10, P 0.0001). The maximum value for group 3, 140 µg g^{-1}, did not exceed the minimum value of group 2, 226 µg g^{-1}.

The levels of zinc in the livers of the control group 1 and the lead dosed group 2 were not significantly different, group 1 mean 173 µg g^{-1} (range 114-206 µg g^{-1}) and group 2 mean 160 µg g^{-1} (range 114-206 µg g^{-1}). There was a significant difference between group 1 and group 3 mean at 242 µg g^{-1} (range 198-259 µg g^{-1}) ($t = 4.27$, df 10, P 0.0016) and also between groups 2 and 3 ($t = 4.95$, df 10, P 0.0006). Kidney zinc levels were higher in group 3 than the control group 1 ($t = 5.52$, df 10, P 0.0003). Mean values and ranges for group 1 and 3 were 62.0 µg g^{-1} (range 54.2-78.1 µg g^{-1}) and 84.9 µg g^{-1} (range 78.1-92.1 µg g^{-1}) respectively. The difference between the control group 1 and group 2 (mean 70.9 µg g^{-1}, range 44.6-90.1 µg g^{-1}) and also between groups 2 and 3 were not significant.

There was no significant difference between the bone levels of zinc in groups 1 and 2, mean and ranges for groups 1 and 2 were 107 μg g^{-1} (range 87.2-132 μg g^{-1}) and 111 μg g^{-1} (range 86.0-136 μg g^{-1}) respectively. There was, however, a significantly higher level of zinc than the controls in group 3: mean 285 μg g^{-1} (range 219-316 μg g^{-1}) ($t = 11.32$, df 10, P 0.0001). The level of zinc in group 3 was also higher than group 2 ($t = 7.18$, df 10, P 0.0001).

Barium test meal taken 10 days after dosing

The X-rays taken 20 minutes after administration of barium sulphate showed radio opaque material in the intestines of the control group of birds and peristalic movement could be clearly seen. Forty minutes after dosing barium sulphate could be seen in the large intestine, ileum and cloaca. Group 3 the combined dosed birds, showed similar alimentary tract activity, except for one female which showed no gut activity after a third X-ray taken 60 minutes later. Three of the birds from group 2, two females and one male, showed no movement of the test meal after 1 hour; two more, one female and one male, had a gizzard and alimentary tract movement considerably slower than the control group. Peristaltic movement could not be detected but radio opaque material could be seen in the duodenal section of the small intestines after 1 hour. The remaining male had normal gut activity.

Although, in general, tissue levels of lead were higher in group 3 than in group 2, the clinical signs of lead poisoning during the 18 day trial were less pronounced in the combined dosed birds, demonstrating a limited protective action of zinc against lead toxicity. Zinc has many therapeutic uses and has been shown to have a protective action against chemical damage in rats (Cerklewski and Forbes 1976), algae (Roderer 1986) and cultures of Englena gracilis (Falchuk et al. 1975). The continued gut activity in group 3 after the tenth day of the experiment when gut activity in group 2 had stopped in all but one bird indicates that, despite higher tissue lead levels in the combined dosed birds, they were able to bind the lead in a less toxic state. It is thought that increased zinc intake can induce both renal and hepatic metallothionein (Bremner 1974) and this could bind up the lead in a less toxic form. Detoxification of lead is most likely to have occurred in the alimentary tract. Cerklewski and Forbes (1976), in part of their study, injected zinc into rats fed on a high lead diet and found no protective mechanism of zinc administered in this way; however, they did find a beneficial effect of feeding a high zinc diet to rats simultaneously fed on high levels of lead.

The daily dietary level of lead in the group 3 birds using a uniform erosion rate of lead of 12.2 mgm and mean daily food consumption was 148 ppm on day 1 to 400 ppm on day 17. Both levels are higher than

zinc similarly calculated. Blood lead levels at post-mortem were mean 418 g/100 ml (range 390-460 µg/100 ml) and mean 401 µg/100 ml (range 360-440 µg/100 ml) for lead fosed and lead zinc dosed birds respectively and were not significantly different (t = 1.0674, DF10, P 0.3109). This indicates that lead absorption in the alimentary canal is not influenced by the presence of higher than normal levels of zinc which is contrary to the findings of Cerklewski and Forbes (1976) whose work showed a decreased level of lead in blood, liver, kidney and tibias of rats. But in their study dietary levels of zinc exceeded those of lead. Continued gut activity in the combined dose group 3 birds would account for the higher erosion of lead in this group.

The highest level of zinc supplementation used by Cerklewski and Forbes (1976) was 200 ppm and this reduced the toxic effect of 200 ppm lead to that of 50 ppm. In my study assuming a uniform erosion rate of zinc of 10.5 mgm per day, the daily dietary level of zinc in group 3 ranged from 120 ppm on day 1 to 328 ppm on day 17. These figures are well below the level of zinc dose feed of 1000 ppm shown to be tolerated by chickens (Mehring et al. 1956) and can be classed as non-toxic. The lower levels of lead in the bone of the combined dose group of birds indicate that either bone has a greater affinity for zinc than lead or that the lead in the soft tissues of the body is not available for absorption. The latter is most likely since the higher tissue levels of lead in the mixed dosed birds were having less influence on the physiological state of the bird than the lower tissue levels of lead in

group 2. In a study by Willoughby et al. (1972), lower levels of lead were found in the bones of young horses when dosed with toxic levels of lead and zinc than lead alone. They also noted that combined lead/zinc dosing gave signs of only zinc intoxication. Different results were obtained in a similar study with pigs in that zinc enhanced the toxic effect of lead when they were both fed at toxic levels together (Hsu et al. 1975). Adminstration of zinc can induce bone resorption in the rat (Yamaguchi et al. 1983) and also promote significant elevation of zinc content of the femur, so a preference of bone for zinc rather than lead cannot be discounted. This may also lead to an elevation of lead levels in the soft tissues.

Zinc is essential to numerous metalloenzymes, one of which is ALAD. This enzyme is involved in haemoglobin synthesis and is a sensitive biochemical indicator of lead toxicity (Weissberg et al. 1971). In a case of human lead poisoning, the administration of Ca EDTA for chelation therapy induced excretion of lead, but further depressed blood ALAD (Thomasino et al. 1977). The activity of the enzyme was restored following oral administration of zinc. It is also believed that loss of zinc during chelation therapy for the treatment of lead poisoning is responsible for the toxic side effects of this drug (Brownie and Aronson 1984).

The examination of duck gizzards in America (Jordan and Bellrose 1951) and Great Britain (Mudge 1983) showed that, of those which contained shot, 80% contained no more than two. In Great Britain the average number of fishing weights ingested by swans has been shown to be between 7 (Birkhead 1982) and 11 (Simpson et al. 1979). This present study has examined the effect of ingesting a realistic number of shot which could occur if zinc is adopted as an alternative to lead as a shotgun pellet on fishing weight. The trial has shown that zinc is unable to stop the progress of lead intoxication but has a limited protective action which may be therapeutically important if the ratio of zinc to lead was increased. But most important this study has shown that there is no potentiation of the effects of lead intoxication.

TABLE 1

Results of liver, kidney and bone analyses of Mallard for lead and zinc expressed in $\mu g\ g^{-1}$ on a dry weight basis.
(mean ± SE)

		Liver		Kidney		Bone	
	Bird No.	Lead	Zinc	Lead	Zinc	Lead	Zinc
ıtrol	1	<5	186	<5	59.6	38.1	118
	2	<5	164	<5	54.2	31.0	102
	3	<5	206	<5	78.1	31.0	102
	4	<5	114	6.1	62.7	46.0	87.2
	5	<5	183	<5	59.9	29.2	98.1
	6	<5	188	10.8	58.0	41.0	106
			173±13.0	8.4±1.1	62±3.3	34.2±3.7	107±6.4
ad dosed	7	108	160	303	74.1	340	124
	8	120	141	344	68.2	226	95
	9	216	206	325	70.1	292	122
	10	210	114	300	44.6	356	136
	11	70.5	190	83.6	90.1	232	105
	12	195	150	244	78.4	353	86
		153±25	160±13	266±13.6	70.9±6.1	299±24	111±7
ad/zinc	13	405	238	400	89.4	112	280
sed	14	414	259	411	92.1	98.4	311
	15	1180	198	521	79.1	68.2	219
	16	498	260	493	82.0	111	290
	17	312	252	398	88.6	140	298
	18	430	249	460	78.1	131	316
		539±130	242±9	447±21	84.9±2.3	110±10.3	285±14

TABLE 2

Weights of zinc and lead fishing weights administered to Mallard and remaining in the gizzard at the end of the experiment. Number of weights in brackets.

	Weight of shot administered to test groups (g)		Weight of shot eroded (g)	
Bird	Lead	Zinc	Lead	Zinc
7	0.510		0.201 (5)	
8	0.492		0.211 (5)	
9	0.520		0.220 (5)	
10	0.487		0.197 (5)	
11	0.477		0.297 (4)	
12	0.500		0.201 (5)	
13	0.481	0.397	0.214 (5)	0.131 (5)
14	0.474	0.401	0.222 (5)	0.198 (5)
15	0.510	0.386	0.234 (5)	0.146 (5)
16	0.531	0.409	0.378 (4)	0.192 (5)
17	0.496	0.403	0.212 (5)	0.211 (5)
18	0.510	0.400	0.217 (5)	0.201 (5)

REFERENCES

Ball, W.L., Sinclair, J.W., Crevier, M. & Kay, K. (1954). Modification of parathion toxicity for rats by pretreatment with chlorinated hydrocarbon insecticides. Can. J. Biochem. Physiol., **32**, 440-445.

Barltrop, D. & Khoo, H.E. (1975). The influence of nutritional factors on lead absorption. Post-graduate Medical Journal 51, 795-800.

Barnett, A., Rattner, J.M., Becker & Tsutoniu Nakatsugawa (1987). Enhancement of parathion toxicity to quail by heat and cold exposure. Pestic. Biochem. Physiol., **27**, 330-339.

Bellrose, F.C. (1959). Lead poisoning as a mortality factor in waterfowl populations. Bull. Ill. St. Nat. Hist. Surv., **27**, 235-88.

Birkhead, M. (1981). How the fishermen kill the swans. New Scient., **90**, 14-15.

Birkhead, M. (1982). Causes of mortality in the Mute Swan Cygnus olor on the River Thames. J. Zool. Lond., **198**, 15-25.

Birkhead, M. (1983). Lead levels in the blood of Mute Swans Cygnus olor on the River Thames. J. Zool. Lond. **199**, 59-73.

Bremner, I. (1974). Heavy metal toxicities. Q. Rev. Biophys., **7**, 75-124.

Brownie, C.F. & Aronson, A.L. (1984). Comparative effects of Ca Ethylenediaminetetraacetic acid (EDTA). Zn EDTA and ZnCa EDTA in mobilizing lead. Toxicol. Appl. Pharmacol., **75**, 167-172.

Cerklewski, F.L. & Forbes, R.M. (1976). Influence of Dietary Zinc on Lead Toxicity in the Rat. J. Nutr., **106**, 689-696.

Dieter, M.P. & Luake, J.L. (1978). Studies on combined effect of organophosphates on carbamates and morsodren in birds. II Plasma and brain chlorinesterase in quail fed morsodren and orally dosed with parathion or carbofuran. Bull Environ. Contam. Toxicol. **19**, 389.

Durham, W.F. (1967). The interaction of pesticides with other factors. Residue Rev. Vol.18, 21-104.

Falchuk, K.H., Fawcett, D.W. & Vallee, B.L. (1975). Competitive antagonism of cadmium and zinc in the morphology and cell division of Euglena gracilis. J. Submicrosc Cytol., **7**, 139-152.

Forbes, G.B. & Reina, J.C. (1972). Effect of Age on Gastro-intestinal absorption (Fe, Sr, Pb) in the Rat. J. Nutr., **102**, 647-652.

Finley, M.T. & Dieter, M.P. (1978). Influence of laying on lead accumulation in Bone of Mallard Ducks. J. Toxic. Environ. Health, **4**, 123-129.

French, M.C. & Haines, C.W. (1987). Investigation into the effects of ingestion of zinc shot by Mallard Ducks (Anas platyrhynchos). Environ. Pollut. **47**, 305-314.

Hsu, F.S., Krook, L., Pond, W.G. & Duncan, J.R. (1975). Interactions of Dietary Calcium with Toxic Levels of Lead and Zinc in Pigs. J. Nutr., **105**, 112-118.

Jordan, J.S. & Bellrose, F.C. (1951). Lead poisoning in Wild Waterfowl. Ill. St. Nat. Hist. Surv. Biol. Notes No.26.

Keplinger, M.L. & Deichmann, W.B. (1967). Acute toxicity of combinations of pesticides. Toxicol. Appl. Pharmacol., **10** 3, 586-595.

Mehring, A.L.Jr., Brumborough, J.H. & Titus, H.W. (1956). A comparison of the growth of chicks fed diets containing different quantities of zinc. Poultry Sci., **35**, 906-958.

McEwen, J.E. (1963). The seasonal incidence of lead poisoning in the North of Scotland. Vet Record Vol.75 No.20, 515-516.

Mudge, G.P. (1983). The incidence and significance of ingested lead pellet poisoning in British wildfowl. Biol. Cons., **27**, 333-372.

Roderer, G. (1986). On the toxic effects of tetraethyl lead and its derivatives on the Chrysophyte Poterioochromonas malhamensis. Ecotoxicol. Environ. Saf., **11**, 277-294.

Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of Mute Swans. Environ. Pollut., **18**, 187-202.

Thomasino, J.A., Zuroweste, E., Brooks, S.M., Petering, H.G., Lerner, S.I. & Finelli, Y.N. (1977). Lead, Zinc and erythrocyte aminolevulinic acid dehydratase: Relationships in lead toxicity. Arch. Environ. Health, **32**, 244-247.

Tompsett, S.L. (1939). The influence of certain constituents of the diet upon the absorption of lead from the alimentary tract. Biochemical Journal, **33**, 1237-1240.

Weissberg, J.B., Lipschutz, F. & Oski, F.A. (1971). aminolevulinic acid hydratase activity in circulating blood cells (A sensitive laboratory test for the detection of childhood lead poisoning). New Engl. J. Med., **284**, 565-569.

Willoughby, R.A., MacDonald, E. & McSherry, B.J. (1972). The interaction of toxic amounts of Lead and Zinc fed to young growing horses. Vet Record 91, 382-383.

Yamaguchi, M., Takahashi, K. & Okada, S. (1983). Zinc induced hypocalcaemia and bone resorption in rats. Toxicol. Appl. Pharmacol, **67**, 224-228.

Chapter 11

Final Discussion

There is little doubt that lead poisoning, following the ingestion of fishing weights, has killed a considerable number of swans in many parts of Britain. East Anglia is no exception. It rates as one of the worst areas in lowland Britain, in that up to 74% of swans found dead and then included in my survey were found to have died of lead poisoning.

The deaths of waterfowl caused by lead poisoning is not a new phenomenon. Ingested lead gunshot has probably always been the prime cause of deaths in ducks, however in recent years lead poisoning due to anglers split shot was shown to be a major mortality factor in the Mute Swan in Britain.

Lead fishing weights have been in use for over 150 years, until their ban in 1987. Surprisingly, they only appear to have caused noticeably substantial deaths of Mute Swans in the past two decades. It has been suggested that the problem began in the late 1950s (Hunt 1977). Prior

to this date it was common practice for anglers to keep their terminal tackle (hooks, weights and float) on a winder ready for use and then return them to the winder at the end of a days fishing. However, using the newly introduced nylon monofilament line, the tendency was to remove the hook and strip off the lead weights, allowing them to fall into the water or onto the river bank. This practice must have contributed to the 250 tonnes of lead weights estimated to have been lost in or near British rivers each year (NCC Report 1981). Birkhead (1982) calculated that this amounted to 2 shot for every 30 cm of river in this country.

Why then do swans eat lead fishing weights? It has been suggested that they do so in mistake for grit needed to aid digestion or accidentally when they attempt to free themselves from line entanglement or when eating bait used by anglers (Sears 1988). Whilst the latter is probably true, the results in Chapter 2 suggest that most weights are eaten by choice.

The 1987 ban on the use of lead fishing weights has generally resulted in an improvement in the situation. The number of swans dying of lead poisoning has declined and blood lead values have similarly fallen (Sears 1988). This implies that swans are killed by lead weights that have been freshly deposited. Previous to the ban, this idea was

supported by evidence of lead poisoning occurring in swans in areas where fishing had only recently been permitted. The following are 2 examples. Lead weights discarded at a pond near Coalville, Leicestershire were responsible for the death of one swan within 2 months of the pond being opened for fishing. Similarly, 3 swans died within 6 months of fishing commencing at the lake at Melbourne Hall in Derbyshire (Perrins 1985).

Evidence from other studies also shows that swans die after eating large fishing weights and ledgers (NCC Report 1981). The size of grit in the one hundred swans sampled in Chapter 2 was smaller than the vast majority of ingested lead weights, suggesting that they select weights which are of a larger size than their favoured grit. I believe this evidence supports the theory that lead weights, when available, are ingested by choice.

During the early 1980s, it was often argued that swans could have been killed by lead which came from petrol engines on boats or from lead in the run off from roads. Both ideas can be discounted. Water Authorities carry out regular checks on river water and they have reported no detectable elevation in lead concentration. Background levels of lead in soil and on vegetation vary considerably and, in some cases, lead levels can be very high. However, swans living and feeding in these areas do not reflect these levels (Chapter 2).

It is true to say that all swans, which I have examined, carry a background level of lead in their tissues, caused by environmental contamination, probably most of which originates from petrol-engined motor vehicles (Chapter 2). However, very high tissue levels of lead in swans are nearly always associated with fishing weights present in the gizzard. Other workers have recorded seasonal variations in the blood lead values of swans from fished areas, unlike levels recorded in unfished waters (Birkhead 1982, Sears 1988).

It has been shown that a breeding population of swans can be reduced on rivers subjected to navigation (Hardman and Cooper 1980), and declining river quality can further reduce a river's value as a breeding habitat (Bacon 1980, Birkhead 1981). The lack of vegetation would undoubtedly make it easier for swans to pick up lead weights. It is also possible that the lack of river vegetation affects a swan in such a way as to make it more vulnerable to lead poisoning, other work has suggested that a diet of vegetable material could have a therapeutic or protective effect against lead poisoning (Jordan & Bellrose 1951).

In vitro, it has been shown that constituents of the swans natural diet have no detectable action when homogenised with a solution of lead (Chapter 7). However, Chapter 7 presents evidence to support the idea that dietary supplements, namely bread and wheat, can play an important role in reducing the effect of lead intoxication.

It is possible that diet may play a supportive role during treatment for lead poisoning, in that bread or wheat would help immobilise lead in the gut and prevent its re-absorption. It is therefore possible that in the field, the swans' diet could be a deciding factor in whether birds survive ingestion of a small amount of lead. This situation could exist in the wild, since swans on many rivers rely heavily on the public to supplement their diet with bread and, in some areas, bread forms a major part of their diet (Birkhead 1982, Sears 1989).

Following the publication of the Nature Conservancy Council Report (1981), the media mounted a prolonged campaign featuring the plight of the lead-poisoned swan. Members of the public were encouraged to report dead or dying swans to interested organisations. Many schools used lead poisoning in swans as a wildlife project and several swan rescue groups were set up in different parts of the country. Despite this extensive media coverage and public awareness, only a small proportion of the approximately 3,700 swans, estimated to die each year from lead poisoning, were notified (Perrins 1985). This leaves a vast majority of bodies unreported to any official organisation. The results of the carcass-recovery experiment (Chapter 2) show that only 35% of the bodies which were returned to their original location were re-reported, but 69% disappeared, all within 12 days.

Lead and calcium are said to follow the same metabolic pathways (Clarke & Clarke 1975) and this link has been used to explain some features of lead poisoning. If this theory is correct then the increased calcium need during the formation of eggshells should provide an ideal test. However, using pigeons the results from Chapter 3 indicate that these metabolic links are difficult to demonstrate in lead-poisoned birds. There was no detectable change in blood lead values during the formation of eggshells, a time of maximum calcium stress in the female bird. The calcium/lead ratio in the bone of the laying birds differed greatly from the calcium/lead ratio in the eggshells produced by these birds. These results indicate either that calcium and lead are not closely metabolically linked, or that calcium for eggshells is almost entirely derived from the diet and not the medullary bone. Both views are counter to popular belief.

Throughout the past 10 years, inexperienced animal welfare groups have treated lead-poisoned swans with little success. In some cases considerable suffering was caused due to lack of veterinary supervision and misuse of drugs. Experiments carried out by Bratton (1981) showed that daily injections of thiamin (Vitamin B_1) was successful in treating lead poisoning. Here was a drug that would be readily available, easy to use, and would overcome many of the problems encountered when in inexperienced hands, after all it is "a naturally

occurring compound, readily enters the cell, is not toxic and efficiently reduces the toxicity of lead" (Bratton 1981). Unfortunately, initial optimism was unfounded, and I am unable to confirm Bratton's treatment, and my study (Chapter 6) has shown little therapeutic value in thiamin administration.

When swans are undergoing treatment for lead poisoning, analysis of the levels of lead in blood and, more importantly, the urine should be undertaken to determine the efficiency of therapeutic action. However, urine collection in birds would be time consuming and difficult, apart from being stressful for the birds. Evidence from Chapter 5 shows that faecal lead measurement may be therapeutically important in that it is an indicator of the body's natural ability to eliminate lead. Treatment for lead poisoning may also enhance this process of gut excretion but I did not test this aspect.

At the present time, when dead swans are categorised as lead poisoned, the criteria used include either tissue lead values, veterinary evidence (ie symptoms) or a combination of both. It is a valid question to ask as to what tissue value should be set and what can we classify as sufficient veterinary evidence to confirm lead poisoning. The usual tissue values are 50 mg/kg^{-1} for liver and 125 mg/kg^{-1} for kidney and these have been used in this thesis and also extensively in

recent literature (Simpson 1979, French 1982, Birkhead 1982, Sears 1988). However, these values should be open to question and provided that there is sufficient other evidence, they could be changed. In Chapter 2, veterinary or post-mortem evidence together with lower than usual tissue lead values were used to place 68 swans in the lead-poisoned category. Without chemical analysis of tissue, diagnosis of lead poisoning from post-mortem symptoms alone would be very difficult, since much of the damage exhibited in the organs of lead-poisoned birds is similar to that of starvation, which could be caused by other events (Wobeser 1981). The deciding factor at post-mortem examination is usually the presence of lead weights in the gizzard, but these may not always be present or detected.

Many swans collide with overhead cables and other obstacles and research has shown that swans which are carrying higher than background levels of lead in their tissue may be more prone to collision accidents. Location of wires and cables over areas of water which are suitable for swans will obviously increase the risk of collision. But results from Chapter 7 show that sublethal lead poisoning affects a bird's ability to function normally, in that landing ability is significantly influenced by lead intoxication. Thus swans, which were classified as having died accidentally by collision, but which had higher than background levels of lead in their tissues, might rightly

also have been classified as lead-poisoned. Perhaps consideration should be given to the introduction of another category when defining lead poisoning in birds:- acute, chronic and contributory.

The analysis of a single tissue for lead may also not give a true indication of body load or effect. Results from Chapter 3 lend weight to this view. The seasonal change in liver lead residues noted in this study also indicates that a degree of caution should be used when interpreting data from birds sampled at different times of the year. The change in liver lead levels was closely linked to the moult and associated zinc metabolism.

One of the many recommendations of the NCC Report on lead poisoning in swans was to encourage research to develop non-toxic alternatives to anglers' lead weights. The search for a suitable alternative for lead gunshot has been underway since the early 1950s (Longcore et al. 1974). Nearly all these alternatives were based on lead, either in combination with or coated with various other metals. All proved to be toxic and were rejected. However, steel gunshot has been successfully developed and is regularly used, particularly in the United States of America. Steel formed the basis of the first fishing weight alternative (Evode) to be tested by me at Monks Wood for possible toxic effects. Tests on other substances followed, tungsten (Sandvik Safeweight, now available as a safe shotgun cartridge pellet), tin

(Thamesley Sureshot) and finally zinc (A M and S Europe and Dinsmore). All proved to be environmentally safe and the findings for one example, zinc, is illustrated in Chapter 9. During this particular study, on ducks receiving what was purported to be a nutritionally balanced diet, it became evident that zinc had a measurably beneficial effect on feather quality. It is important to consider the possibility that any substitute fishing weight, once ingested, could modify the toxicity of the ubiquitous lead weight. This situation could readily occur now that other materials, one being zinc, have been adopted as a suitable fishing weight.

Simultaneous ingestion of lead and zinc (Chapter 10) demonstrated a mildly protective action of zinc against lead toxicity. The birds in this study were dosed with equal proportions of lead and zinc, but as more zinc is used and less lead is available, due to its ban, the possibility exists that the levels ingested may be of considerable therapeutic importance. More importantly, this study has shown that there was no potentiation by zinc of the effects of lead toxicity.

Some aspects of this study, namely the post-mortem results and environmental survey, complement or confirm previous research in the Thames basin by Birkhead (1982) and Sears (1988). Other aspects, such as dietary constituents, excretion, effect on locomotion and calcium metabolism have to my knowledge not been reported in birds.

REFERENCES

Bacon, P.J. (1980). Status and dynamics of a mute swan population near Oxford between 1976-1978. Wildfowl **31**: 37-50.

Birkhead, M.E. (1982). Causes of mortality in the mute swan on the River Thames. J. Zool., **198**, 15-25.

Bratton, G.R., Zmudski, J., Bell, M.C. & Warnock, L.G. (1981). Thiamin (Vitamin B_1) Effects on lead intoxication and deposition of lead in tissues: Therapeutic Potential. Tox. Appl. Pharm. **59**, 164-172.

Clarke, E.G.C. & Clarke, M.L. (1967). Garners Veterinary Toxicology. 3rd ed. New York: Williams and Wilkins.

French, M.C. (1982). Lead poisoning in Bewick Swans. BTO News, No.121, 1.

Hardman, J.A. & Cooper, D.R. (1980). Mute swans on the Warwickshire Avon - a study of a decline. Wildfowl **31**: 19-36.

Hunt, A.E. (1977). Lead poisoning in swans. BTO News **90**: 1-2.

Longcore, J.R., Locke, L.N., Bagley, G.E. & Andrews, R. (1974). Significance of lead residues in Mallard tissues. Special Scientific Report Wildlife No.182. Washington D.C.

Nature Conservancy Council (1981). Lead poisoning in swans. London NCC.

Perrins, C.M. (1981). Mortality of mute swans. Unpub. Rep. to the working group on lead poisoning in mute swans.

Sears, J. (1989). Feeding activity and body condition of mute swans Cygnus olor in rural and urban areas of a lowland river system. Wildfowl, **40**, 88-98.

Sears, J. (1988). Regional and seasonal variations in lead poisoning in the mute swan Cygnus olor in relation to the distribution of lead and lead weights in the Thames Area, England. Biological Conservation **46**, 115-134.

Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of mute swans. Environ. Pollut. **18**: 187-202.

Wobeser, G. (1981). Diseases of wild waterfowl. Plenum Press ISBN 0-306-40746-7.